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CONTENTS

No. 1 (February 1951)

Original Articles: PAGE

432. The condition and average live and carcass weights of 'wastage' animals in west Wales (1943–46). R. Phillips 1

433. The design and use of a milking machine for sows. D. M. Smith, W. G. Whittleston and J. Allen 31

434. The bacteriological quality of National Dried Milk. A. T. Parsons and J. R. Fraser 34

435. Keeping quality and raw-milk grading. II. The effect of storage on the results of keeping quality and dye tests. R. T. Eddison, O. Kempthorne, Zena Hosking, H. Barkworth, C. P. Cox and A. Rowlands 43

436. The estimation of citric acid in dairy products. J. Babad and N. Shtrikman 72

437. Copper and iron in dairy produce. Improved methods of analysis. Dawn R. Perrin, F. Ruth Lightfoot and G. M. Moir 77

438. The viscosity temperature coefficient of homogenized milk. J. E. Caffyn 95

439. The rate of creaming of raw cows' and buffaloes' milk. A. H. Fahmi 106

Reviews of the Progress of Dairy Science:

Section A. Physiology of dairy cattle. Part II. Physiology and biochemistry of rumination. E. C. Owen 113

No. 2 (June 1951)

Original Articles:

440. Determination of lactose in dairy products. J. Babad and A. Grunpeter 147

441. Equipment for individual-quarter machine milking. H. S. Hall . 151

442. Variations in composition of cows' milk during a period of 12 weeks. J. Featherstone, C. D. Rickaby and A. J. Cavell 155

443. Keeping quality and raw-milk grading. III. Comparison of keeping quality tests and dye tests in milk control (results of a field trial). A. Rowlands and Zena Hosking 166

444. The production of antibiotics by streptococci. A. Hirsch and Dorothy M. Wheater 193

445. The differentiation of the lactic streptococci and their antibiotics. A. Hirsch and Elisabeth Grinsted 198

446. A note on the inhibition of an anaerobic sporeformer in Swiss-type cheese by a nisin-producing streptococcus. A. Hirsch, Elisabeth Grinsted, Helen R. Chapman and A. T. R. Mattick 205

Reviews of the Progress of Dairy Science:

Section B. Bacteriology and mycology applied to dairying. E. R. Hiscox and A. Hirsch 207

No. 3 (October 1951)

Original Articles: PAGE

447. Milk yield in relation to infection with *Streptococcus agalactiae*. D. H. McLeod and S. M. Wilson 235

448. Machine milking rate and mastitis. F. H. Dodd and F. K. Neave . 240

449. The deterioration of milking rubbers. I. The effect of micro-organisms. N. J. Berridge 246

450. The effect of the presence of air on the heating of full milk bottles by steam. C. C. Thiel and H. Burton 251

451. Biochemical and serological properties of some citric acid fermenting streptococci from milk and dairy products. Per F. Swartling . . 256

452. Bacteriological tests for milk sampled during distribution to the consumer. A. Rowlands and Zena Hosking 268

453. Nutritive value for rats of sow's milk in comparison with cow's milk. R. Braude, K. M. Henry, S. K. Kon and K. G. Mitchell . . 283

454. Bacteriological aspects of the reworking and blending of stored butter. E. R. Hiscox and A. G. House 291

455. The ripening of Stilton cheese: changes in the volatile acid content. E. R. Hiscox, J. Harrison and J. Z. Wolf 296

456. The calcium and phosphorus of Cheddar and Stilton cheese. I. Cheddar and Stilton cheese of normal manufacture. II. The distribution of calcium between the crumb and coat of Stilton cheese. (The late) E. C. V. Mattick 305

Reviews of the Progress of Dairy Science:

Section D. Nutritive value of milk and milk products. S. K. Kon and K. M. Henry 317

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CONTENTS OF THE JANUARY ISSUE

Editorial, by THE PRESIDENT. Seventh Annual General Meeting.

Laboratory Control of Processing and Manufacture, by J. G. DAVIS and T. R. ASHTON.

The London Dairy Show, by E. CAPSTICK.

The Production of Sterilised Milk, by F. PROCTER.

Detergents and Their Use in Dairy Plant, by R. J. MACWALTER.

Laboratory Control, by A. L. PROVAN.

Sectional Notes.

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CONTENTS OF VOL. III, No. 4, DECEMBER 1950

Kerala Varma, H. Laxminarayana and K. K. Iya. Studies on the Spore-Forming Bacteria in Milk. I. The Incidence and Distribution of Spore-forming Aerobes in Milk and Farm Materials.

(Miss) Jer S. Khambatta and Noshir N. Dastur. Changes in the Chemical Composition of Milk During Souring.

A. Roy, S. N. Luktuke, S. Bhattacharya and P. Bhattacharya. Studies on the Reducing Substances of Semen. Part II. Relation of Sperm Concentration and Semen volume to Fructose Content, Fructolysis and Methylene Blue Reduction Time.

C. V. Chandrasekhar. Effect of Refrigeration on the Micropopulation of Raw and Pasteurised Milk.

V. Sreenivasa Murthy, V. K. N. Nambudripad and K. K. Iya. Studies on Vitamin B_{12} Activity of Milk.

636.2:658

432. THE CONDITION AND AVERAGE LIVE AND CARCASS WEIGHTS OF 'WASTAGE' ANIMALS IN WEST WALES (1943–46)

BY R. PHILLIPS
Department of Animal Health, University College, Aberystwyth

(With 7 Figures and 14 Maps)

Certain particulars of the wastage from the dairy and breeding herds of the west Wales region have been obtained from an analysis of the statistics relating to the sales of cows, cow-heifers and bulls through the twenty-five collecting centres of the fat stock scheme of the Ministry of Food. The scheme, which has been in operation since 1940, has effected the purchase for slaughter of fat stock in accordance with their grade and weight. The twenty-five collecting centres of this region draw their supplies from a number of the surrounding parishes which have been allocated to them.

The following key map of the region gives the location and an outline of the collecting area of each centre.

Map 1. The twenty-five collecting centres of west Wales. (1), weekly marts; (2), fortnightly marts.

The amount of trade passing through each centre naturally varies with the size of the area, an aspect which has been noted in previous analyses of the individual mart records (Phillips & Davies (1), Phillips (2), Phillips & Brown (3).

The region is a self-contained peninsula consisting of the three counties of Cardiganshire, Carmarthenshire and Pembrokeshire. It has a 'farming area' of approximately

1¼ million acres (one-third of the whole of Wales and Monmouthshire), which is apportioned into nearly 20,000 holdings. It consists of approximately 70% crops and grass and 30% rough grazings, and carries an average population of about 300,000 head of cattle, with numbers of sheep, horses, pigs and poultry, etc. The agriculture of the region has been described up to 1939 by Ashby & Evans(4); the cultivated pastures have been described by Stapledon(5) as being of the *Agrostis*/Wild White clover complex, and soil chemists have shown that the region is generally deficient in both CaO and P_2O_5.

In the past, the region was concerned with the rearing of store-cattle, with some fattening in the better lowland areas, but during recent decades, and particularly during the war years, it has become an important milk-producing area, and it is generally agreed that during the 8 years 1940–8 wastage in cattle in this region was fairly characteristic of dairy herds in the country. Owing to the loss of the mart records for the 3 years 1940–2, the survey has had to be limited, except when otherwise stated, to the years 1943–6. Sanders(6), Hammond(7) and Roberts(8) have estimated from the examination of the registers of milk-recording societies an annual wastage of nearly 30%, and have concluded that the average milking life of a dairy cow is about 3½ years. Pettit(9) and Howell(10), on the other hand, have obtained estimates from farm surveys which show a 25% annual wastage or an average effective life of 4 years per cow. Smith(11), Marley(12) and Phillips(13) have separately computed from official statistics (quarterly returns) for in-calf heifers an annual replacement figure of 700,000–800,000 or approximately 25% for the national dairy and breeding herd, and Phillips(13) has reached a similar conclusion in the case of the individual Welsh counties.

The west Wales region, by this method however, gives less reliable estimates than the others because of the practice of selling some cows of all grades outside the region. It has been shown, on the other hand, that even figures given by the national herd sometimes fail to tally. During the years under review in this paper, for example, when the national herd was fairly stationary in size, the annual intake of first-calf heifers was between 700,000–800,000 head of cattle as against 500,000–600,000 recorded as slaughtered by the Ministry. Only some of the 200,000 not included in these records can be traced to knackeries. Pettit(9), Howell(10) and Phillips(14) have computed that deaths from other causes account for 1–2% of most dairy herds.

The records of the collecting centres for cows and cow-heifers account for 6–7% of the population in west Wales, i.e. for about 25% only of the annual wastage. Incomplete as this figure is, however, it nevertheless provides valuable and interesting information. One other modifying factor is that practically all the cows offered to the Ministry during this period were accepted for slaughter; only a very small number were 'refused stores' during the 4 years 1943–6.

Cows 'accepted on the hoof' are purchased on live weight after being relegated by the official graders of the Ministry of Food first to one of the four grades: special (S), A, B or C, according to their degree of fleshiness and fatness. Further subdivisions of grades A, B and C have no bearing on this discussion. Those cows which are in too poor a condition for grading are accepted on dead weight, but may subsequently be condemned if found unfit for human consumption after slaughter. The same applies to damaged (W) and casualty cows.

This discussion is concerned for the most part with cows, cow-heifers and bulls with occasional reference to the total cattle sales which also include fat steers and heifers.

The total cattle sales (1940–8)

The gross number of cattle of all descriptions sold to the Ministry of Food for slaughter from this region is shown in Fig. 1 for each year from 1940 to 1949.

Fig. 1 shows an annually increasing sale of fat cattle from 1940 to 1946 followed by a decline in 1947 and in 1948. This annual trend is similar to that described by Phillips (13) in the sale of calves. An average of nearly 24,000 head of cattle was sold each year, which represents 8% of the cattle population (4 June) and 17% of the breeding female population. As already mentioned, this low rate of disposal is explained by the fact that alternative methods of selling stock are available.

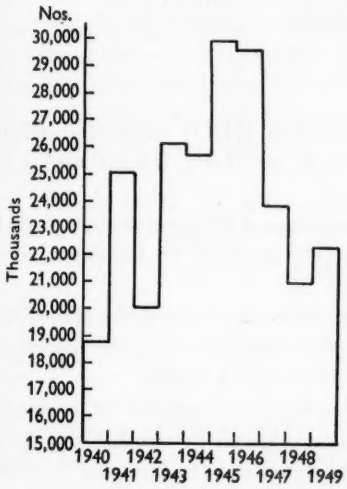


Fig. 1. Annual gross sales of all cattle to Ministry of Food in west Wales during the 10 years 1940–9.

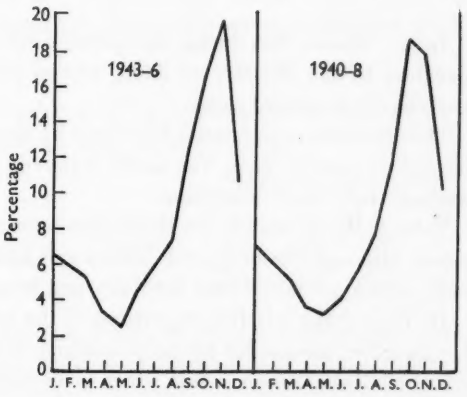


Fig. 2. Seasonality of sales of all graded fat cattle in west Wales.

The seasonality of the sales of all fat cattle in west Wales is shown in Fig. 2 for the periods 1940–8 and 1943–6.

Fig. 2 shows that the main deliveries occurred during the autumn months, and they agree with the general production of beef in Great Britain as described in the *Economic Series* (15). The 2 months of glut deliveries (October and November) account for 36% of the annual total, whilst during the 4 spring months (March–June) only 16% of the annual total were delivered. In other words, most of the cattle sold for slaughter are the immediate products of the grasslands of the region.

There has been a marked change in the relative proportions of the spring and autumn deliveries of cattle during the whole of the last decade, as shown in Table 1.

Table 1. *The relative proportions (percentage) of the annual sales of total cattle in spring and autumn* (1940–8)

Season	1940	1941	1942	1943	1944	1945	1946	1947	1948	1940–8	1943–6
Spring (Mar.–June)	22·1	24·4	20·5	21·5	17·3	14·0	9·3	8·6	7·4	15·8	15·3
Autumn (Sept.–Dec.)	52·9	37·9	39·0	49·6	58·7	58·0	67·2	72·2	74·5	57·8	58·9

The autumn glut and the spring scarcity have become more acute with the advancing years, and by 1948 the autumn deliveries are ten times as great as those in the spring.

The volume of the trade in each of the categories for graded cattle for the 4 years 1943–6 are given in Table 2.

Table 2. *The numbers and percentage contribution of each category of graded cattle* (1943–6)

Categories	Total numbers	Percentage contribution		Gross sales of graded and ungraded cattle
Steers	23,674	27·4	58·5	
Heifers	26,889	31·1		
Cow-heifers	4,197	4·8	41·5	111,171
Cows	28,177	32·8		
Bulls	3,650	3·9		
	86,587	100·0		

Table 2 shows that during the period 1943–6, a total of 111,171 cattle were sold for slaughter to the Ministry of Food, and of these 86,257 or 77·8% are included in this analysis of the graded cattle.

The difference is accounted for chiefly by the non-grading of some cattle, but partly by the loss of mart records. The latter, however, have little influence on the nature of the wastage trade under discussion.

Table 2 also shows that in this region cows are the most numerous of the graded categories, although the beef cattle (steers and heifers) outnumber the cows, cow-heifers and bulls, which are taken from the dairy and breeding herds of the region.

In Table 2 the relative proportions of the various categories of graded cattle accepted for slaughter are similar to the proportion of cattle slaughtered by the Ministry for the whole of Great Britain, a fact which gives additional significance to the total 'cow beef' in home-killed beef supplies.

Part I. The sales of graded cows, cow-heifers and bulls (1943–6)

The proportions of these three categories of 'wastage cattle' and the average live weights for each category have been drawn together in Table 3.

Table 3. *The numbers, percentage contribution and average live weight of the graded cows, cow-heifers and bulls* (1943–6)

Category	Total numbers	Percentage contribution	Average live weights (lb.)
Cows	28,177	78·2	1100·0 (9 cwt. 3 qr. 8 lb.)
Cow-heifers	4,197	11·6	962·3 (8 cwt. 2 qr. 10 lb.)
Bulls	3,650	10·2	1516·0 (13 cwt. 2 qr. 9 lb.)
Total	36,024	100·0	1126·0 (10 cwt. 0 qr. 6 lb.)

It will be noted that cows form the largest category and represent 87% of the female sales. Their average live weight is 138·7 lb. (nearly 1¼ cwt.) heavier than that of the cow-heifers. Graded bulls average 13½ cwt. in live weight, but while the grading of all 28,177 cows is available, only 75% of the accepted bulls and cow-heifers have been grouped in this way. The percentage contribution of each grade is given in Table 4.

Table 4. *The grading of cows, cow-heifers and bulls* (1943–6)

	Percentage grading			
Category	S (Special)	A	B	C
Cows	10·4	13·8	42·1	33·7
Cow-heifers	1·6	14·8	62·8	20·8
Bulls	3·0	50·2	37·5	9·3
Total	9·0	16·8	43·6	30·6

Cows form the greatest proportion of grade S and cow-heifers the lowest. Bulls are the highest in grade A. A consideration of the three categories separately shows that: 53% of the total bull sales are in grades S and A; 24% of the total cows fall into these two groups, and in the case of cow-heifers 16·4%. This reveals the distressing fact that over three-quarters of the cows and the cow-heifers fall into the two lower grades, and that as much as one-third of the cows and one-fifth of the cow-heifers fall into grade C.

Grading gives some indication of size and live weight of the animals. Table 5 gives the average live weight for each grade and indicates the relationship between condition (grade) and body size (live weight).

Table 5. *The average live weight of each grade of cows, cow-heifers and bulls compared with grade* A (*estimated at* 100 *in each case*)

	Average live weight (lb.)					Comparisons: grade A = 100			
Category	S	A	B	C	Average	S	A	B	C
Cows	1101·4	1201·0	1108·0	1047·5	1099·7	92	100	92	87
Cow-heifers	1102·1	1057·3	968·7	887·7	966·9	104	100	88	84
Bulls	799·3	1667·1	1434·8	1302·6	1521·9	48·5	100	85	78

The similarity of weight between cows and cow-heifers of grade S is due chiefly to the small difference between their average ages. Bulls of grade S are markedly lighter than their neighbours of grade A; this is probably because they are very young bulls in excellent condition which were reared for service but which failed for some reason to qualify for a breeding licence. The outstanding superiority in weight of grade A bulls (and to a less degree, of grade A cows) is due to their having reached maturity and their prime condition. Cows of grade B are typical dairy cows of the autumn, and characteristic products of this region. Grade C cows are autumn cullings from the dairy herds, and other aged, worn-out cows. Their size may vary from that similar to the average grade A and B cow, to the smaller built Jerseys and Ayrshires of dairy breed, but their weight and condition are uniformly low.

Table 5 (where grade A = 100) also shows that the average live weights of grades A–C are in the ratio of 100:90:80 respectively. An improvement on the condition of the 'wastage' animals of grades B and C would mean that the supply of home-produced 'cow beef' was materially increased.

The seasonality of the sales of graded cows, cow-heifers and bulls

It has already been shown in Fig. 2 that deliveries of fat cattle in west Wales occur mainly during the autumn months. This is also true for each of the three categories separately, as shown in Fig. 3, where the monthly average live weights are superimposed on the corresponding supply graph.

As expected, there are very high autumn peaks and low spring troughs in cow and cow-heifer deliveries. The two months October and November account for over one-third of the annual total. In the same general pattern the average live weights of the two categories are at their highest in late summer and autumn, and at their lightest in spring. The seasonal variations in body size are not very marked, and in both cases the extreme differences are less than 56 lb. The cows are the heaviest throughout.

With bulls, the peak deliveries occur in June, although the highest average monthly live weight is in April. The average live weights of bulls during the first part of the year are materially higher than for the second half.

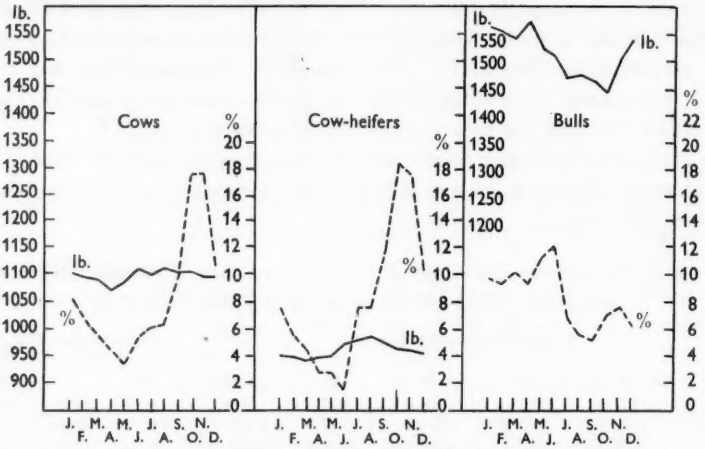


Fig. 3. Seasonality of the sales of graded cows, cow-heifers and bulls (1943–6), together with monthly average live weights. ——, monthly average live weight (lb.); – – –, percentage monthly distribution.

These differences can be explained; the cows and cow-heifers are sold for slaughter 'off the grass' probably without preparation. Bulls, on the other hand, are kept indoors all the year round and are always in fairly good condition. Their peak periods of service activities are in June and January to correspond with peak calvings in October and March (17). After their normal 3-year breeding life they are fattened and sold off, and the young bulls take their places.

It is fairly easy to fatten dual-purpose bulls because they are already mature and in good condition. Advantage is taken of the lag after the January peak service to fatten old bulls, and they are therefore delivered in their greatest numbers between April and June, when they are at their highest live weight. Another reason for these high spring deliveries is that bulls, like rams, are not very acceptable to the butcher except in times of scarcity.

The seasonality of the sales of the four grades of cows, S, A, B and C (1943–6)

Graphs of the seasonal deliveries of all grades and their average monthly live weights are shown below.

All four graphs are of the usual pattern with peak deliveries in autumn (October for grades S, A and B, and November for grade C), but it is noticeable that as the grade deteriorates the autumn peak increases. The reverse is found in the spring troughs where the supplies diminish as the grade deteriorates.

There is a July hump in the graph for cows of the S grade, but it is much less evident in grades A and B, and is absent in the grade C graph. The summer gap in the lowest grade (C) is wider than it is in the others.

The graphs show that for every month of the year, cows of grades S and B are very similar in average live weights, while cows of grades A and C are at approximately equal distances above and below the respective levels of grades S and B. The monthly average live weights of all grades are at their lowest in spring and highest in late summer and autumn.

The histograms in Fig. 4 illustrate the relationship between spring and autumn deliveries of the four grades of cows, and the details are given in Table 6.

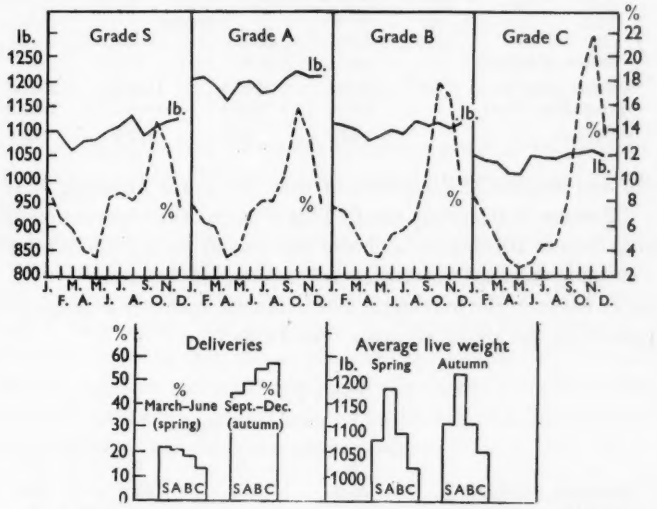

Grade S
Grade A
Grade B
Grade C
lb.
%
J. M. M. J. S. N. J. M. M. J. S. N. J. M. M. J. S. N. J. M. M. J. S. N.
F. A. J. A. O. D. F. A. J. A. O. D. F. A. J. A. O. D. F. A. J. A. O. D.
Deliveries
March–June (spring)
Sept.–Dec. (autumn)
Average live weight
Spring
Autumn
S A B C

Fig. 4. Seasonality of graded cows; percentage monthly—average monthly live weights. – – – –, percentage; ——, average weight.

Table 6. *Spring and autumn deliveries of cows of the four grades expressed as a percentage of the annual totals in each case, together with spring deliveries expressed as a percentage of autumn.*

Season of deliveries	Percentage grades S	A	B	C
Spring (Mar.–June)	21·9	21·3	18·5	13·9
Autumn (Sept.–Dec.)	44·5	47·9	54·5	61·7
Spring deliveries as percentages of autumn	49·1	44·5	34·0	22·5

Table 6 shows that in grade S, for example, the spring deliveries amount to 21·9% and the autumn ones to 44·5% of the annual total of cows of this grade. The table shows that as the grade deteriorates from S to C the spring percentages diminish and the autumn ones increase. As regards the lower section of Table 6, spring deliveries as a percentage of autumn become smaller from grade S to grade C, and therefore the proportion of cows in grade C increases as the year advances. The probable reasons for this seasonal change in the sales of cows for slaughter are: (*a*) there are alternative markets for the lower grade

cows in spring because of the demand for 'barren cows' for fattening elsewhere on summer grass, but not in the autumn; (*b*) some of the low-grade cows in autumn are no doubt the aged spring calvers, which, after milking through the summer, are culled and sold in the fall. Calving is always highest in spring in this region (13), and this becomes more pronounced with advancing age of the cows (16). Irrespective of grade, it was found that 26·7% are sold in spring and 37·8% in the autumn.

The average spring and autumn live weights of all four grades of cows are compared in detail in Table 7*a*, *b*.

Table 7*a*. *Comparison of the average live weights* (*in lb.*) *of the four grades of cows in spring and autumn* (1943–6)

	Grades			
Season of deliveries	S	A	B	C
Autumn (Oct.–Dec.)	1118·8	1213·4	1112·1	1052·8
Spring (Mar.–May)	1075·5	1182·1	1089·7	1020·0
Difference	43·3	31·3	22·4	32·8

The average live weights of all grades of cows are lower in spring than in autumn, although the difference is relatively small. This confirms the observation that cattle are lighter in spring than in autumn (14), and also that casualties in farm livestock are highest in spring (17), probably because the winter diet is often inadequate.

The differences between the maximum and minimum monthly average live weights are of course greater than the above averages. See Table 7*b*.

Table 7*b*

	Grades			
	S	A	B	C
Maximum difference in monthly average live weight (lb.)	69·0	58·9	36·7	45·9
Maximum difference as percentage of average live weight	6·2	4·9	3·3	4·4

In general, the higher the grade the greater the monthly or seasonal differences. As a percentage of the live weight these differences are small.

Although the differences in the seasonal live weights are small, there is a large difference in the proportions of the monthly deliveries (Fig. 4). It is obvious therefore that body weight and condition do not alone determine the monthly rate of disposal; there are other factors in operation such as seasonal price levels of cow beef, the season of calving, the state of lactation and milk yields, available food supplies and future prospects of improvement in condition. Aged spring calvers will milk reasonably well on summer grass although in relatively lean condition, but the cost of winter fattening is high in relation to increase in weight and improvement in grade; consequently culling occurs when their use as milk producers has come to an end. On the other hand, young cows of the dual purpose breed will more readily qualify for grade S and can therefore be sold at times other than during the autumn glut.

So far, consideration has been given to the 4-year averages of all four grades. The data deserve individual annual attention, however.

Annual deliveries and average live weights of wastage cows (1943–6)

Both the numbers and the proportions of the four grades change from year to year, but in the main the grading remained unchanged during this period. The order of importance of grades S and A changed over from 1943 to 1945 but 1946 restored the *status quo*.

Table 8. *The annual numbers and the proportions of the four grades of cows in* 1943, 1944, 1945 *and* 1946

	Grades (%)					Numbers				
Year	S	A	B	C	B+C	S	A	B	C	Total
1943	7·3	17·4	47·5	27·8	75·3	392	942	2567	1500	5401
1944	11·9	11·7	41·3	35·1	76·4	841	827	2920	2478	7066
1945	14·4	12·0	39·8	33·8	73·6	1163	966	3214	2716	8059
1946	7·2	15·1	41·3	36·4	77·7	547	1157	3157	2790	7651
1943–6	10·4	13·8	42·1	33·7	75·8	2943	3892	11858	9484	28177

The highest percentage of the cows are always in grade B followed by grade C, and these two grades account for 75% of the cows and are therefore most characteristic of the region's cattle.

The rising number of cattle sold per year shows that the breeding of dairy cattle, with emphasis on summer milk production, has proved a satisfactory alternative to the traditional rearing of store-cattle, although it is the latter which thrive best on the type of pasture found in this region, and designated by Stapledon(5) as 'second-rate'. We may conclude, therefore, that the only means of immediately improving the milk output in the region would be to increase the number of milch cows, since there is evidently less hope of increasing the milk yield of each cow. The latter could only be achieved by an improvement of the pastures and the methods of conserving fodder.

The annual average live weight of each grade of cow

Table 9. *The annual average live weight of cows per grade* (1943–6)

	Average live weights (lb.)				
Year	S	A	B	C	All grades
1943	1100·9	1211·6	1123·2	1063·2	1120·3
1944	1099·7	1205·3	1104·5	1048·0	1095·9
1945	1086·6	1199·1	1103·8	1043·9	1092·4
1946	1137·1	1190·6	1102·6	1042·5	1096·4
1943–6	1101·4	1201·0	1108·0	1047·5	1099·7

Table 9 shows a remarkably constant average live weight, although there is a slight tendency for the body size to diminish during the 4 years. The figures for grades A, B and C in 1943 may represent the averge live weights realized by the last batch of the pre-war rearings.

The above figures describe roughly the limits in average body weights of the cow population of the region. Although there has been an increase in the number of Friesian, Ayrshire and Jersey cows in recent years, the above figures disclose practically no change in the average live weights of cows of each grade and even of all the cows which were sold to the Ministry of Food during this period. It is therefore evident that the region as a whole

can produce and maintain a particular body size in the non-pregnant female breeding stock, which varies only slightly from year to year.

The relationship between the annual numbers of graded cows, cow-heifers and bulls sold to the Ministry of Food and their respective populations (On 4 June) in the region

As previously stated, the cows, cow-heifers and bulls passing through the collecting centres represent only a portion of the annual wastage in the dairy and breeding stock of this region. The cow-heifers have been included with the cows, because many of them have at some time or other been included in the category described in the Agricultural Returns as 'Cows and heifers in milk'. Table 10 gives the numerical relationship of the two categories.

Table 10. *The relationship between the annual numbers of cows, cow-heifers and bulls sold for slaughter, and their respective populations (Agricultural Returns, 4 June)*

	Cows and cow-heifers			Bulls		
Year	No. graded	Total population	Graded (%)	No. graded	Total population	Graded (%)
1943	6,002	133,961	4·5	815	4,739	17·2
1944	8,104	139,125	5·8	1,069	4,959	21·6
1945	9,419	139,280	6·8	984	4,935	20·0
1946	8,849	140,461	6·3	782	4,993	15·7
1943–6	32,374	552,827	5·8	3,650	19,626	18·6

It is seen that the graded 'wastage' cattle sold for slaughter are a low percentage of the June population. This is because alternative markets are available. If the annual replacement figures for the female breeding stock and for bulls are 25 and 33% respectively, then the proportions in the above table indicate that 25% of the wastage cows are sold for slaughter within the region, whilst 60% of the wastage bulls are sold for the same purpose.

Differences within the region

It has already been shown by Phillips & Davies(1) and by Phillips & Brown(3) that the breeding season and body size of farm animals within the region of west Wales vary considerably. Thus the extent of autumn calvings (or winter conceptions) in cows and heifers gradually declines from the western coastal areas of Pembrokeshire to the eastern boundaries of Cardiganshire and Carmarthenshire. The average carcass-weight of all categories of sheep (rams, ewes, sheep and lambs); the average live weight of fat steers and heifers, the concentration of fat cattle, pigs and rabbits as well as the proportion of winter milk all show a similar decline.

It is to be expected therefore, that both in seasonality and in average live weight the 'wastage' stock (cows, cow-heifers and bulls) would show similar trends.

(*a*) *Differences per county*

That these trends exist is shown by the following comparisons for the groups of collecting centres allocated to each county in the region. The details of these averages are given in Table 11.

Table 11. *Average live weights of each category in the three counties* (1943–6)

County	Average live weights (lb.)		
	Cows	Cow-heifers	Bulls
Cardiganshire	1095·3	935·5	1496·4
Carmarthenshire	1088·0	954·2	1510·8
Pembrokeshire	1115·8	978·2	1549·7

Pembrokeshire produces the heaviest cattle in all three sections, although the difference is relatively small. The difference between the Pembrokeshire average and the lower averages of the other two counties is as follows: 27·8 lb. for cows; 24·7 lb. for cow-heifers and 53·3 lb. for bulls, representing only 2–3% of the average live weight in each case.

Differences between the average live weights of the four grades in the three categories for each county are shown in Table 12.

Table 12. *The average live weight per grade of cows, cow-heifers and bulls* (1943–6)

County	S	A	B	C
		Cows		
Cardiganshire*	1086·5	1196·0	1098·5	949·1
Carmarthenshire	1094·7	1200·0	1103·3	1043·5
Pembrokeshire	1130·9	1203·7	1117·4	1052·4
		Cow-heifers		
Cardiganshire*	1036·0	1031·4	968·0	904·7
Carmarthenshire	1073·3	1069·9	958·2	885·7
Pembrokeshire	1110·9	1058·1	980·5	886·5
		Bulls		
Cardiganshire*	840·0	1641·4	1389·0	1169·1
Carmarthenshire	945·5	1642·4	1576·1	1302·6
Pembrokeshire	679·0	1726·9	1482·7	1333·8

* Centres Aberystwyth and Ystrad are the only centres in Cardiganshire contributing to the grades of cow-heifers and bulls.

The maximum, by however narrow a margin, is reached by Pembrokeshire in eight of the above twelve series. This county provides the lightest grade S bulls and it takes second place to Carmarthenshire in Grade A cow-heifers and to Cardiganshire in grade C cow-heifers. It also takes second place to Carmarthenshire in grade B bulls.

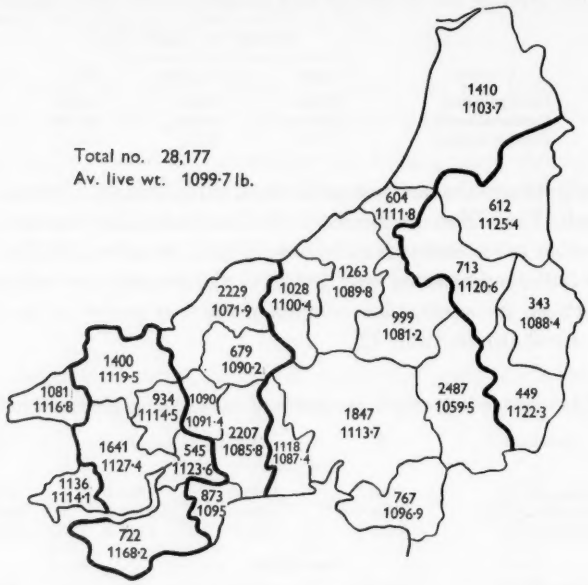
(*b*) *Differences per collecting areas*

Comparative figures of 'wastage' cattle for each collecting area (1943–6). The following maps give the numbers and average live weights of cows, cow-heifers and bulls for each of the twenty-five collecting areas in the region. On each map a thick outline of four groups of centres are shown. The significance of this partition will be dealt with later in the paper.

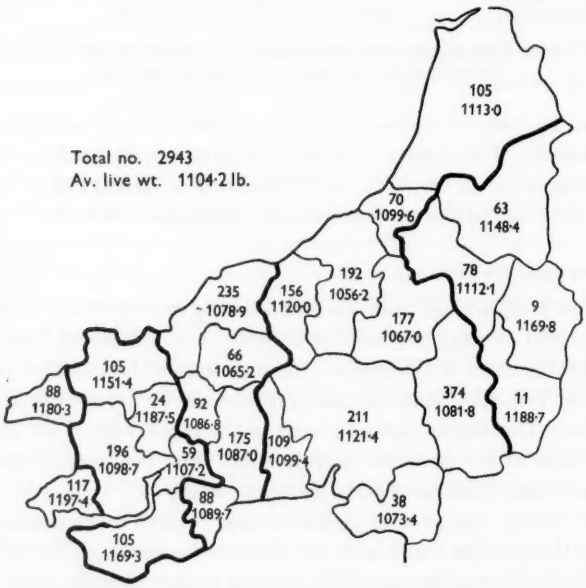
Map 2. *All cows.* The central dairying areas of the region supply the greatest numbers of cows, while those of the heaviest weights come from the west Pembrokeshire area; heaviest of all are those from the Pembroke centre itself.

Map 3. *Grade S cows.* The general picture of supplies and average weights is the same as in Map 2, but the heaviest come from the Hasguard collecting area, whilst those of the Llangadock and Llandovery centres of the eastern boundaries are equal in body size to Pembroke, Hasguard and Croesgoch centres.

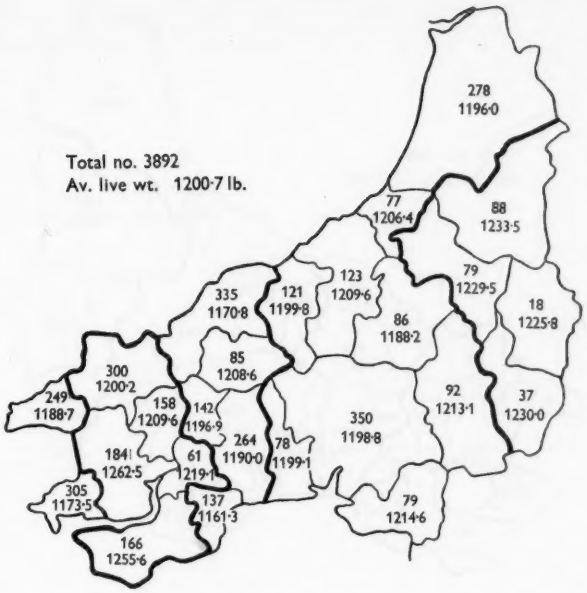
Map 4. *Grade A cows.* There is much greater uniformity throughout the region in these



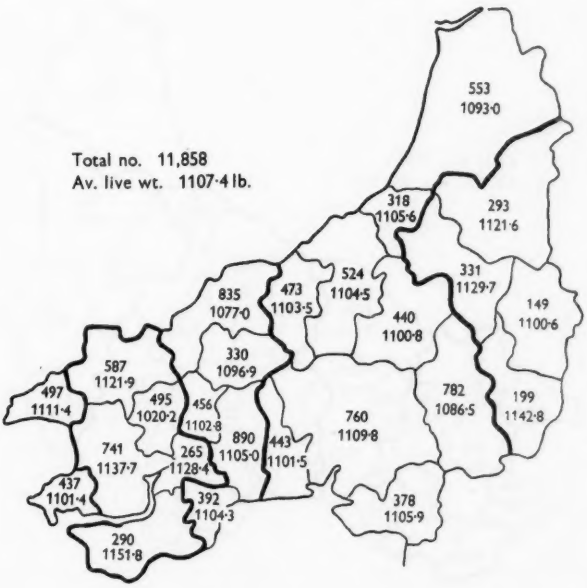
Map 2. Average live weights (in lb.) and numbers of all graded cows (1943–6) per centre.



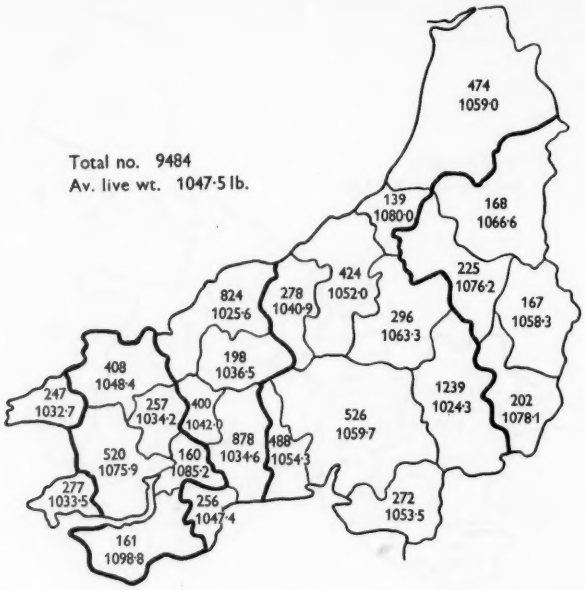
Map 3. Average live weights (in lb.) and total numbers of grade S cows (1943–6) per centre.



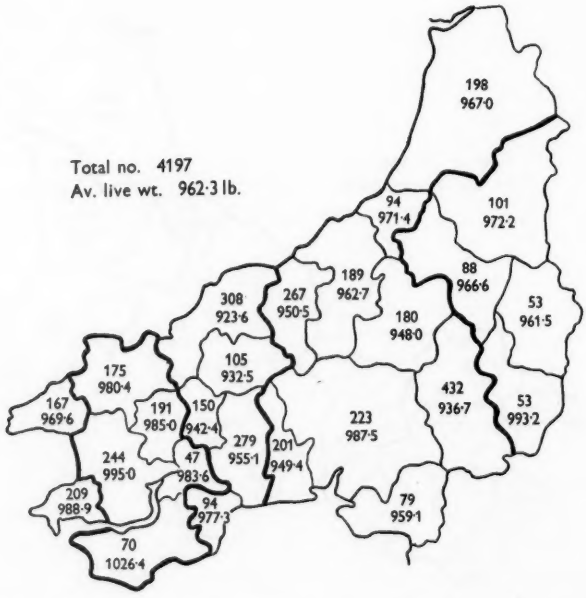
Map 4. Average live weights (in lb.) and total numbers of grade A cows (1943–6) per centre.



Map 5. Average live weights (in lb.) and total numbers of grade B cows (1943–6) per centre.



Map 6. Average live weights (in lb.) and total numbers of grade C cows (1943–6) per centre.



Map 7. Average live weights (in lb.) and total numbers of cow-heifers (1943–6) per centre.

Map 8. Average live weights (in lb.) and total numbers of bulls (1943–6) per centre.

Map 9. Average live weights (in lb.) of all graded cows and total numbers of cows (1943–6) in each of four groups of centres.

mature cows in good condition. The heaviest of all come from the Pembroke collecting area, but closely followed by Tregaron, Llandovery and Llangadock centres of the eastern sections. The lightest cows of this grade are from the Kilgetty area in south Pembrokeshire.

Map 5. *Grade B cows.* The picture remains fairly uniform; the lowest weights come from Cardigan (1077 lb.) and the heaviest from Pembroke (1151·8 lb.). Maximum difference = 74·8 lb.

Map 6. *Grade C cows.* The lightest cows are from Llandilo (1024·3 lb.) and the heaviest again from Pembroke (1098·8 lb.), showing a maximum difference of 74·5 lb.

Map 7. *Cow-heifers* (average of all grades). Pembroke is the only centre supplying cow-heifers of over 1000 lb. average live weight. Cardigan and Crymmych are the two lowest, the maximum difference being 102·8 lb.

Map 8. *Bulls.* The Pembroke centre again contributes the highest (1654·8 lb.) and Llandilo the lowest (1420·4 lb.) with a difference of 234·4 lb. between them.

The correctness of the grading

The average live weights of each centre for each of the four grades of cows, for each of the 4 years 1943–6, has been submitted to statistical analysis and the standard deviation from the means of the above averages have been calculated.

It was found that twice the standard deviation was invariably greater than the maximum differences between the average live weights and the mean, which indicates that the grading was satisfactorily performed within each grade of cows. This is in agreement with Warman's conclusions(18).

Inter-centre comparisons

A comparison of the various centres has been made from the point of view of the differences between their respective average live weights and the mean of the average live weights of the region, for each grade, each year, and for the 4-year averages. As a result, it was found possible to group the twenty-five centres into two groups as follows:

Group 1. Where the average live weights are invariably above the mean value.

Group 2. Where the average live weights are invariably below the mean value.

These two groups were then subdivided according to location and the difference between the average live weights and means in each case, thus making four groups in all. These are illustrated in Map 9.

Average live weights in groups of centres

Grouping of centres

Map 9 shows that there is a fair regularity in the arrangement of the groups of centres from the western coastal areas to the inland easterly ones.

Group 1 occupies the coastal plateau of west Pembrokeshire except for the Hasguard and Croesgoch areas and consists of Pembroke, Narberth, Haverfordwest, Clarbeston and Mathry.

Group 2 lies in a belt of country about 20 miles wide behind group 1, running along the Pembrokeshire, Carmarthenshire and Cardiganshire borders and including the upland areas of the Precelly countryside. This covers the centres of Cardigan, Crymmych, Clynderwen, Whitland and Kilgetty.

Group 3, a wide belt of country, includes the coastal centres of Ystrad and Aberystwyth and also Croesgoch and Hasguard farther inland. Most of this belt forms the core of the dairying area of the region.

Group 4, covering the easterly foot-hills of the region, comprises the four centres of Tregaron, Lampeter, Llandovery and Llangadock.

Certain particulars of the 'wastage' cows in each group of centres are given in Table 13.

Table 13. *The average live weights of all grades of cows in each group of centres, together with the percentage of cows in each grade* (1943–6)

Total no. of cows	Group	Grades (average live weights in lb.)					Grading (%)			
		S	A	B	C	All cows	S	A	B	C
5,242	1	1,130·6	1,227·0	1,131·8	1,064·7	1,128·2	9·3	16·6	45·5	28·6
7,078	2	1,082·3	1,182·0	1,095·3	1,034·3	1,083·8	9·3	13·6	41·1	36·0
13,740	3	1,097·1	1,194·8	1,101·5	1,045·3	1,094·4	11·9	13·4	40·8	33·9
2,117	4	1,134·8	1,231·5	1,124·4	1,070·7	1,117·1	6·6	10·5	45·9	36·0
28,177	Region	1,101·4	1,201·0	1,108·0	1,047·5	1,099·7	10·6	13·8	42·0	33·6

Table 13 indicates that in the central parts of the west Wales region (groups 2 and 3) the cows are slightly below the regional average live weight in all grades, but in both the western and eastern extremities they are slightly above. This is reminiscent of the maps showing the average carcass weights of veal calves(2), in which the central areas were again lower than the extremities of east and west. In the case of the veal calves it was thought that the traditional husbandry methods accounted for this variation, and farmers of the central area were less concerned with good rearing than their neighbours of the eastern and western centres. It is highly probable that these factors also account for the varying average weights of fully grown cows, although the heavier annual rainfall (70 in.—Fidler and Morgan)* in the belt of land covered by group 2 centres may also have some bearing on the matter.

Table 13 also shows that group 4 supplies the heaviest cows of grades S, A and C, but that group 1 supplies the heaviest in grade B. This group also has the highest average. It is interesting to note that although group 4 shows the lowest total number and the poorest grading, it has an average live weight which is surprisingly high in view of the fact that the land which it covers is higher in elevation than that of groups 2 and 3.

On the other hand, only large animals with well-developed barrels thrive on the rough, coarse fodders of the eastern foothills. The central area immediately to the west, which forms part of the groups 2 and 3, is the most intensive dairying part of the region, and the smaller cow suited to dairying has almost invariably been fed with purchased food concentrates with a view to obtaining the maximum milk yield. (Even spring calvers are optimistically fed in this way in the hope of a higher winter milk yield.)

A further influence on the choice of breed in group 4 centres is the popularity of Herefords in the adjacent Breconshire borders. The evident success of the latter for store-cattle rearing (to which the eastern area is traditionally devoted) has been shown by the recent preference for this breed in the coastal centres of Pembrokeshire, where Welsh Blacks (Castle Martin) were formerly popular. Exceptions, however, are found in the two peninsular centres of Croesgoch and Hasguard, where the average live weights for all cows was lower than in neighbouring centres. The same low standard was found here in the case of

* M.Sc. Theses, University of Wales, 1948 and 1949.

summer suckling lamb (3), and there is little doubt that the relative severity of the weather in this exposed area is largely responsible.

On the whole, however, the differences in average live weight from group to group are not very pronounced. Grade S shows a variation of 52·5 lb., grade A 49·5 lb.; grade B 36·5 lb. and grade C 36·4 lb., making an average difference of 44·4 between the extremes.

The average live weights of graded cow heifers and graded bulls per group of centres

Table 14. *Average live weights (lb.) of the four grades of cow-heifers and bulls in the above four groups of centres* (1943–6)

	Cow-heifers						Bulls					
Group	Total nos.	S	A	B	C	All grades	Total nos.	S	A	B	C	Average A, B and C grades
1	727	1098·7	1088·8	994·5	903·0	991·1	392	594·0	1761·0	1506·0	1416·5	1640·0
2	628	1042·0	1049·1	960·0	873·8	951·6	533	824·4	1647·1	1459·6	1289·0	1527·0
3	1690	1111·1	1048·8	959·5	884·6	961·6	1679	942·4	1651·3	1411·6	1267·0	1529·4
4	106	1036·0	1063·0	989·8	920·5	977·3	113	700·0	1645·3	1435·3	1361·3	1485·2
Region	3151	1102·1	1057·3	968·7	887·7	966·9	2717	799·3	1667·1	1434·8	1302·6	1542·3

This table presents many interesting features. Table 13 (cows) has shown that there is a higher average live weight in groups 1 (west) and 4 (east) than in groups 2 and 3. The difference in live weights between each group and the adjacent neighbour is about 40–50 lb. in each case. In the case of the cow-heifers (Table 14) the total numbers involved are considerably fewer, but apart from the S grade, the two extreme groups (1 and 4) are higher by only 20–30 lb. than the two central groups. Bulls (Table 14) are again relatively few, and there is a much greater variation in weight. As previously observed, bulls in grade S are the young, light ones and cannot really be classed with mature bulls. In grades A, B and C, group 1 centres are invariably higher than the adjoining group 2 by over 100 lb. It is group 4 which contributes the lightest bulls in grade A, but in grade C group 4 bulls have higher averages than the two central groups. Group 4 bulls of grade B are also heavier than those of group 3.

Significance of the difference between the four groups of centres

An attempt has been made to determine whether the average differences in the average live weights of all grades of cows between the four groups of centres as shown in Map 9 are statistically significant.

According to Paterson's (19) method of calculating the value of 't', it has been shown that there is always a significant difference at the 5% level, and in many cases at the 1% level between groups 1 and 2 and between groups 3 and 4, but this is not the case between groups 2 and 3 or between groups 1 and 4.

The difference is less obvious in the case of cow-heifers and bulls because the numbers of animals involved are smaller, and also because there is greater variation in body size between individuals in these two categories.

Further expressions of the differences between the groups of centres as previously defined are shown in (1) the percentages grading per group, (2) average monthly percentage sales and live weights.

(1) *Percentage grading per group*

The details are given in Table 15.

Table 15. *Numbers and percentage grading per group*

Category	Group of centres	Numbers					Percentages			
		S	A	B	C	Total	S	A	B	C
Cows	1	489	869	2,378	1,506	5,242	9·3	16·6	45·5	28·6
	2	656	963	2,903	2,556	7,078	9·3	13·6	41·1	36·0
	3	1,637	1,838	5,605	4,660	13,740	11·9	13·4	40·8	33·9
	4	161	222	972	762	2,117	6·6	10·5	45·9	36·0
	Region	2,943	3,892	11,858	9,484	28,177	10·6	13·8	42·0	33·6
Cow-heifers	1	25	97	450	155	727	3·4	13·3	62·0	21·3
	2	2	83	393	150	628	0·4	13·2	62·5	23·9
	3	19	283	1,059	329	1,690	1·1	16·7	62·7	19·5
	4	1	4	77	24	106	1·0	3·8	72·6	22·6
	Region	47	467	1,979	658	3,151	1·5	14·8	63·0	20·7
Bulls	1	24	207	122	39	392	6·1	52·8	31·1	10·0
	2	18	235	225	55	533	3·4	44·1	42·2	10·3
	3	32	892	617	138	1,679	1·9	53·1	36·8	8·2
	4	1	34	57	21	113	0·8	30·1	50·5	18·6
	Region	75	1,368	1,021	153	2,717	2·7	50·4	37·6	9·3

The comparatively small numbers of cow-heifers and bulls in group 4 make it inadvisable to consider the figures as significant. There is, however, a tendency for the grading to deteriorate from group 1 to group 4 and particularly from group 1 to group 2, as shown by the following table which gives the sums of the two percentages S+A and of B+C respectively.

Table 16. *The sums of grades S and A and of grades B and C of Table* 5

Group	Cows		Cow-heifers		Bulls	
	S+A (%)	B+C (%)	S+A (%)	B+C (%)	S+A (%)	B+C (%)
1	25·9	74·1	16·7	83·3	58·9	41·1
2	22·9	77·1	13·6	87·4	47·5	52·5
3	25·3	74·7	17·8	82·2	55·0	45·0
4	18·1	81·2	4·8	95·2	30·9	69·1

It is now evident in all three categories of wastage cattle that the grading is poorer in group 2 than in group 1 (cf. live weights, Tables 13 and 14). In general, group 3 is also slightly better than group 2, but group 4 is definitely poorer than all the other groups.

This is to be expected, since the fodder supplies in the areas of group 4 cannot possibly be as well harvested as in group 1, but it is surprising that there is such a small difference between their average live weights (Tables 13 and 14).

In the case of all grades of cows, group 4 is slightly heavier in average live weight (although statistically not significant) than group 1. The order is reversed in the case of the cow-heifers except in grade C, where again group 4 is heavier than group 1. In the case of the bulls, however, there is a marked difference in favour of group 1.

It is suggested that the mature cows in group 4 are bigger framed than the others because in the foothill areas the environment is less satisfactory. This may be an illustration of the application of Bergman's Rule to domestic animals, although in the light of Huxley's definition thereof(20), it appears to be unlikely.

Conditions in the centres of group 4 are not suitable for the production of body fat, and consequently the grading of cows in this group is the least satisfactory.

On the other hand, the mild conditions obtained in group 1, which allows for a long grazing season, would be suitable for body-fat production as is reflected by better grading and heavy weights.

Whatever the reason, it has been shown that there is a statistically significant difference between the average weights of all the grades of cows in the adjoining groups 1 and 2 and it is not easy to explain why. This is supported in many instances by similar differences between the respective groups in cow-heifers and bulls.

The seasonality for all cattle of the average live weights per grade and of the delivery for each group of centres

Fig. 5 illustrates percentage of cattle per group delivered during the autumn months (September–December), and also the monthly average live weights for each grade in the four groups of centres.

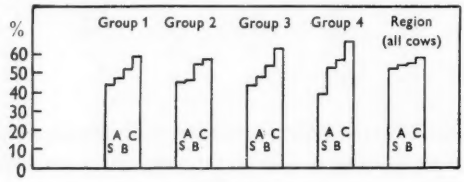


Fig. 5*a*.

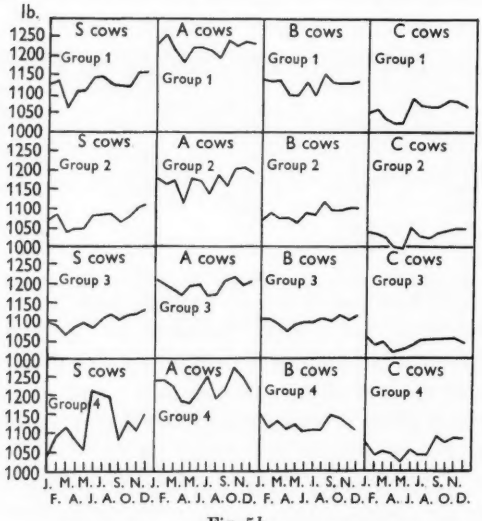


Fig. 5*b*.

Fig. 5. (*a*) Cows sold in autumn (September to December) as percentage of the annual totals (1943–6) for each grade and per group of centres. (*b*) Monthly average live weights of four grades of cows for each group of centres (1943–6).

The histograms in Fig. 5 show that the percentage of cattle relegated to the poorest grade C gradually increases from group 1 to group 4. Group 4 shows not only the highest

autumn deliveries of grade C, but also the lowest percentage deliveries of grade S. In all four groups of centres there is an increase in the percentage delivered as the grade deteriorates, and this is most pronounced in group 4.

The graphs of monthly average live weights per grade for each group show that invariably the lightest are in spring and the heaviest in autumn.

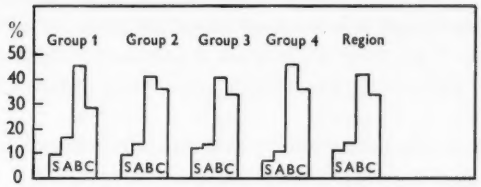


Fig. 6*a*.

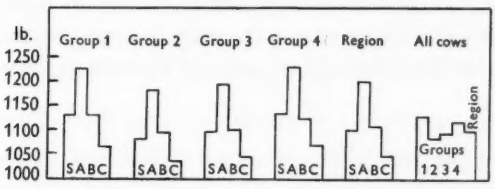


Fig. 6*b*.

Fig. 6. (*a*) Percentage grading per group of centres. (*b*) Average live weights (lb.) for each group of centres.

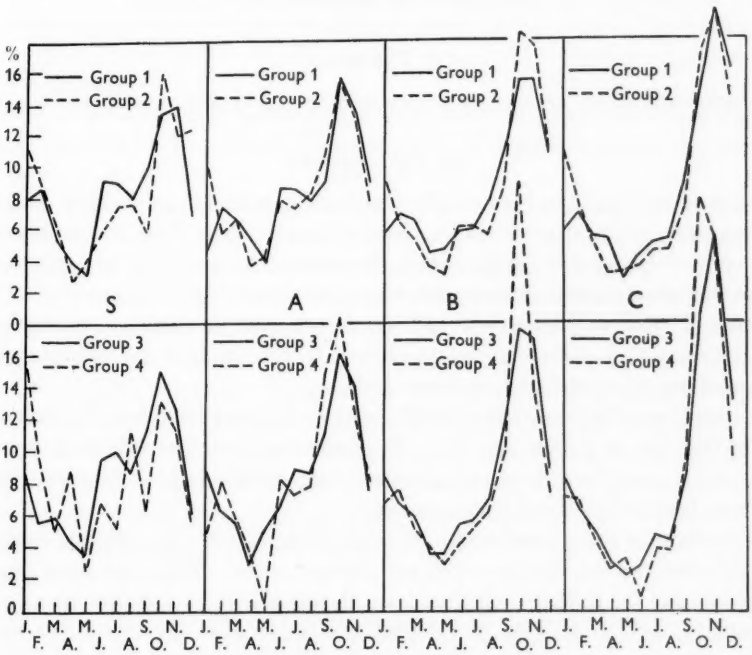


Fig. 7. Percentage monthly distribution of sales of cows for each grade per group of centres (1943–6).

Comparing the various groups, it is seen that for almost every month the averages of group 1 are heavier than those of groups 2 and 3 in all grades. Group 2 shows the lowest monthly averages throughout; group 4 has much smaller numbers and is much more erratic.

The histograms in Fig. 6 give (*a*) the percentage of all cattle delivered by each group to each grade; (*b*) the average live weights of the four grades in each group.

It should be noted that grade B is the most important grade for all four groups, and that the percentage in grade C increases while grade S decreases from group 1 to group 4. The general picture is that the average live weights are lower in groups 2 and 3 then in groups 1 and 4.

The percentage delivered to each grade by each group from month to month is illustrated in the graphs of Fig. 7.

There is a marked regularity in the percentage monthly deliveries for each grade in all the groups. The main feature illustrated is one that has already been shown, viz. that the percentages of autumn deliveries increase as the grade deteriorates and also to a lesser extent as the conditions change from the mild sea areas of the west to the colder climates of the east.

Part II. The ungraded wastage cattle—cows, cow-heifers and bulls

There are occasions when cows presented at the collecting centres are not accepted by the Ministry of Food 'on the hoof' because their live condition disqualifies them for any of the grades S, A, B and C. They are, however, usually accepted on a dead-weight or carcass basis and are classified chiefly as *Rejects* but sometimes as *Casualties*.

(1) *The Rejects*

These are cattle which are in very poor condition and therefore fall below the grade C.

(2) *The Casualties*

These cattle are casualties because of some accident, ailment or deformity, and may be brought in for slaughter at any time irrespective of mart dates. This scheme provides for the salvaging of damaged or partly damaged carcasses. The latter may be condemned, but the wholesome parts are paid for in accordance with the scale of prices fixed by the Ministry. In the case of 'Rejects', however, which are found unfit for human consumption after slaughter, payment is made only for the hide. In this analysis, no separate record of carcasses ultimately condemned has been made.

Since many casualties are strong healthy animals in good condition, the average dead weight of this class is greater than that of the rejected class. The difference, however, is not very pronounced because young animals are also included apart from old, low-conditioned cows that have suffered accidents, etc.

There is also one other small group of cattle brought into the collecting centres, viz. the young cows and cow-heifers which are 'refused stores'. These are promising cattle, which would pay for further feeding and are therefore sent back to the farms or to the store-cattle market. During the period 1943–6 very few cows and cow-heifers came into this class although many maiden heifers and some steers were 'refused stores'.

Gross numbers of graded and ungraded cattle of the three categories

The relative numbers of each group are shown in Table 17. The table shows that one-third of the cows, one-fifth of the bulls but only 9·2% of the cow-heifers accepted for slaughter by the Ministry were ungraded, and that 9·6% of the total females were cow-heifers. The cow group is obviously the most important.

Table 17. *The gross numbers and proportions of graded and ungraded cattle* (1943–6)

	Graded	Ungraded	Total	Ungraded (%)	Approximate ratio graded to ungraded
Cows	28,177	15,086	43,263	34·8	2 : 1
Cow-heifers	4,197	428	4,625	9·2	10 : 1
Bulls	3,650	947	4,597	20·6	4 : 1
Total	36,024	16,461	52,485	31·3	2¼ : 1

The classification of ungraded cattle

The ungraded cattle of each category can be further grouped into Rejects, Casualties and Condemned. The gross numbers and percentages are shown in Table 18.

Table 18. *The classification of ungraded cattle and the percentage of cattle in each section*

	Nos. ungraded cattle			Percentages				
	Rejects	Casualties	Condemned	Rejects	Casualties	Condemned	Total nos.	Percentage
Cows	9,797	1,896	3,393	65·0	12·6	22·4	15,086	91·6
Cow-heifers	157	251	20	36·7	58·5	4·8	428	2·7
Bulls	198	718	31	20·9	75·7	3·4	947	5·7
Totals	10,152	2,865	3,444	61·6	17·5	20·9	16,461	100·0

The relative proportions of each class in each category are widely different. Two-thirds of the ungraded cows are Rejects and one-fifth Condemned with only a small percentage (12·6%) of Casualties.

In the cow-heifer and bull groups, the greatest proportions are Casualties with only a very small proportion Condemned. Three-quarters of the bulls are Casualties, and Rejects one-fifth; while one-third of the cow-heifers are Rejects.

A possible explanation of these differences is that in this relatively 'Clean Area' as far as attestation is concerned, the Rejects are not likely, for the most part, to be tubercular—although undoubtedly there are cases of this disease amongst cattle.

The high percentage of Rejects is a reflexion of the foods and feeding rather than of the breed, type and age, because the cattle of the region are still predominantly dual purpose (Shorthorn and Welsh Blacks).

In the cow-heifers, the vast majority of the Casualties are due to calving troubles, and the Rejects are merely those in low condition. In the bulls, the high percentage of Casualties is very likely due to accidents resulting from viciousness and the fairly high Rejects (20·9%) may be those typical dairy bulls which cannot readily be fattened.

The low percentage of Condemned in the two younger groups (cow-heifers and bulls) in comparison with Condemned cows (22·4%) may be a reflexion of the increasing incidence of disease, etc., with age.

The average dead weight of Reject and Casualty cattle

The average dead weights of the Reject and Casualty cattle are given in the following table. It will be noted that no data have been obtained separately for the condemned

carcasses, because the latter are merely part of the former in the case of partly damaged carcasses. In any case, it is unlikely that even wholly condemned carcasses differ very much from those shown below.

Table 19. *The average carcass weight (lb.) of the Reject and Casualty cattle*

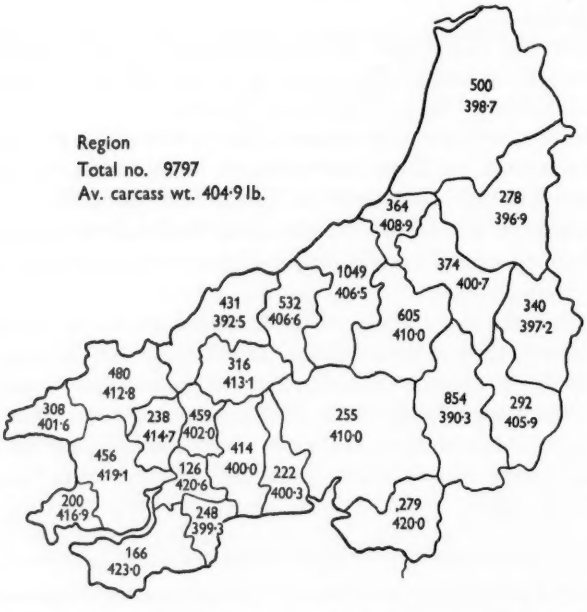
	Average dead weight (lb.)		Difference (lb.)
	Reject	Casualty	
Cows	404·9	447·5	42·6
Cow-heifers	339·6	414·1	74·5
Bulls	512·8	573·1	60·3

In the above three categories the carcasses of Casualty animals are about 0·5–0·75 cwt. heavier than those of the Reject animals.

Whilst the Reject group consists almost entirely of animals in a very low condition, the Casualties consist of a mixture, very many of which may be animals in good condition, which have met with some accident, or even immature cow-heifers and bulls which are merely small in size. It is, therefore, surprising that in view of all these considerations, the differences between the two averages are so small.

The estimated dead weight of cows, cow-heifers and bulls of grade C calculated from the average live weights in Table 5 (Part I) compared with the average dead weight of the Reject animals of Table 19 shows that the Rejects must be considerably lighter than the grade C animals. This supports the general view that the grading has been satisfactorily performed by the graders.

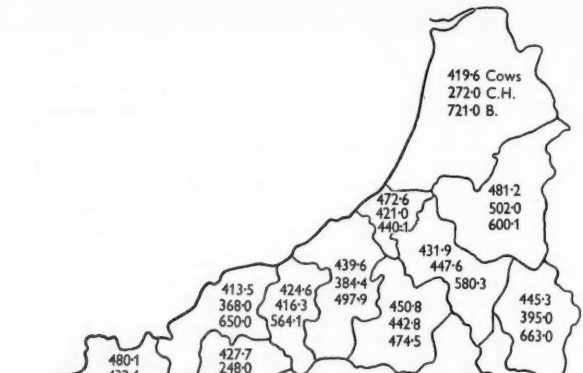
The distribution and average carcass weight of the Rejects and Casualties of the three categories are shown in Maps 10–14.



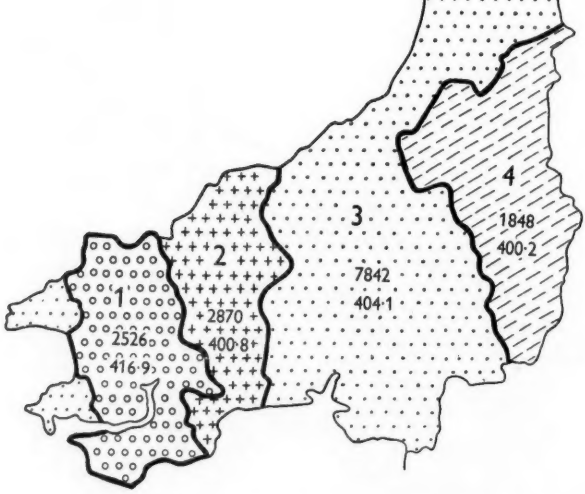
Map 10. Numbers and average carcass weight of Reject cows per centre (1943–6).

Map 11. Numbers of Casualty and Condemned cows per centre (1943–6).

Map 12. Average carcass weight of Reject cow-heifers and bulls (1943–6).



Map 13. Average carcass weight of Casualty cows, cow-heifers and bulls.



Map 14. The four groups of centres with total numbers of the ungraded cattle and average carcass weight of Reject cows.

Map 10 shows that the average dead weight of the Reject cows is fairly uniform throughout the region and there is only 33·3 lb. between the lightest (Llandilo 390·3 lb.) and the heaviest (Pembroke 423·0 lb.).

The total numbers, however, show a much wider difference between the lowest (Pembroke 166) to the highest (Llandyssul 1049).

The number of Reject cows in the large Carmarthen centre is low at 255, but it should be remembered that this is a more intensive dairying area and is therefore well covered by dealers in barren cows.

There was a total number of 9797 Reject cows, which represents 22·6% of the total cows sold to the Ministry of Food included in this investigation.

Map 11 gives the total numbers of Casualty and Condemned cows per centre and this, together with the number of Rejects shown in Map 10, gives a fairly complete picture of the trade in ungraded cows.

Apart from three centres (Lampeter, Carmarthen and Haverfordwest) the map shows that there are more Condemned cows than Casualties. This is also true for the region as a whole, since there are included 3393 Condemned cows and 1896 (or 56%) Casualties.

The centres with the highest and lowest numbers of Casualty cows are Haverfordwest (191) and Llangadock (7) respectively, while for the Condemned cows Llandilo has the highest figure (311) and St Clears the lowest (61).

Map 12 shows the average carcass weights of Reject cow-heifers and bulls. The figures for each of them show much wider variations than those for cows (Map 10). For instance, the figures for cow-heifers in Mathry (278·4), Tregaron (290·0), are low compared with Llangadock (394·0) and Lampeter (389·0).

In the case of bulls, the range is 352·7 lb. in Cardigan and 401·0 lb. in Clynderwen, as compared with 687·6 lb. in Hasguard and 619·3 lb. in Mathry and Llangadock.

The total numbers, however, are relatively small since, during the whole 4 years (1943–6), there were only 157 cow-heifers and 198 bulls of the Reject class.

Map 13 serves as a record of the average carcass weight of all casualty cows, cow-heifers and bulls considered as one group.

The number of animals involved is relatively small and therefore no very great importance can be attached to them. It may be noted in passing, however, that (*a*) in two centres only (Tregaron and Lampeter) the cow-heifers are heavier than the cows; (*b*) the heaviest animals are all found in the western section.

The heaviest of all are an average of 805·2 lb. at Croesgoch for 13 Casualty bulls and 802·0 lb. for 12 Casualty bulls at Narbeth.

At the other end of the scale, Llandilo has an average of 377·1 lb. for 51 Casualty bulls and Llangadock 363·3 lb. for only 3 bulls.

Map 14 illustrates the partitioning of the region into the four groups of centres referred to in Part I and shows that group 3 is the most important numerically; it contains a greater number of ungraded cattle than the other three groups of centres put together. However, each group has a sufficient number to ensure the reliability of the above average carcass weight figures.

Details of the numbers and percentages are shown in Table 20.

The table shows that in the better areas of the west coast (group 1) there are fewer Rejects and more Casualty animals than in the adjoining group 2. Groups 2 and 3 of the more intensive dairying areas have a higher proportion of Rejects and Condemned than the other two groups of centres.

Table 20. *The numbers of Reject, Casualty and Condemned cows in each of the four groups of centres together with the percentages* (1943–6)

Group of centres	Total numbers			Percentages		
	Rejects	Casualties	Condemned	Rejects	Casualties	Condemned
1	1477	500	549	58·5	19·8	21·7
2	1868	270	732	65·1	9·4	25·5
3	5168	930	1744	66·0	11·8	22·2
4	1284	196	368	69·5	10·6	19·9
Region	9797	1896	3393	65·0	12·6	22·4

On the other hand group 4, lying towards the eastern borders of the region, has the highest percentage of Rejects but the lowest percentages of both Casualties and Condemned.

It is inadvisable to read too much into the tables, but they tend to support the previous conclusions with regard to the four parts of this region.

Average dead weights of Reject and Casualty animals for groups of centres

Table 21. *The average dead weight for groups of centres*

Group	Centres	Cows		Cow-heifers		Bulls	
		Reject	Casualty	Reject	Casualty	Reject	Casualty
1	Mathry, Clarbeston, Haverfordwest, Pembroke and Narberth	416·9	472·9	330·6	420·0	552·5	697·6
2	Cardigan, Crymmych, Clynderwen, Whitland and Kilgetty	400·8	429·7	336·2	373·4	507·6	684·7
3	Aberystwyth, Ystrad, Llanybyther, Llandovery, Newcastle Emlyn, Carmarthen, Llandilo, Kidwelly, St Clears, Croesgoch and Hasguard	404·1	440·0	340·1	409·6	491·6	519·4
4	Tregaron, Lampeter, Llandovery and Llangadock	400·2	442·4	364·0	461·3	546·6	572·3
	Region	404·9	447·5	339·6	414·1	512·8	573·1

It will be remembered that in the case of the graded cows, the average figures for group 1 were significantly higher than groups 2 and 3, but that group 4 was nearly equal to group 1.

Apart from a few exceptions, group 1 ungraded cattle are again heavier than those of groups 2 and 3. There is also a regular rise to group 4, although not in all cases.

Condemned meat

A total of 581·34 tons of cattle carcass meat was condemned during the 4-year period under review in the whole region, or an average annual figure of 145·3 tons. The amount varies from centre to centre, but its measure for the four groups of centres is shown in Table 22.

Table 22. *Relation between condemned meat and ungraded cattle per group of centres*

Groups of centres	Total no. of ungraded cattle	Total condemned meat (lb.)	Condemned meat per ungraded beast (lb.)
1	3,565	288,891	81
2	3,098	275,627	89
3	7,840	599,654	76
4	1,958	138,137	69
Region	16,461	1,302,309	80

It is surprising to find how near to the average (80 lb.) each group comes.

DISCUSSION

A number of useful facts of practical importance have been discovered from the analysis of the records of the sales of wastage animals during the 4 years 1943–6 through the twenty-five collecting centres of the Ministry of Food in west Wales.

In general terms, a description of the average body size and condition of the dairy and breeding stock of the region has been presented. In some respects the picture appears to be unsatisfactory, since over one-half of the wastage cows slaughtered are in the two lowest categories. It is suggested that in the main this is a reflexion of the quality of the pasturage and particularly of the winter food supplies. It seems that considerably more cow beef could be produced by improving the condition of these wastage cattle and it is known that this can be achieved by the improvement of the pastures and methods of fodder conservation. The seasonality of production follows the usual pattern of an autumn glut and a spring scarcity. An interesting light is thrown on the point under discussion by the partition of the region into four areas according to the body size of the cows.

It has previously been shown that the climatic conditions in west Pembrokeshire are milder than elsewhere in the region and it was expected that body size would fall with decreasing suitability of the climate from west to east. It is true that the condition of the animals as determined by their grading percentages, tends to substantiate this theory, but in respect of the body size it was disclosed that the central area of the region produced the lightest cows, whilst the eastern region produced cows which were equal in average live weight to those of the most favoured areas of west Pembrokeshire. This same trend had previously been found in the average carcass weight of veal calves but not in the case of fat lambs.

The application of Bergman's Rule to domestic animals has been suggested as an explanation. There may also be other reasons such as the ultimate effect of the rearing of the calves. Another reason may be that a more intensive summer milk production in the central area would tend to reduce body size. On the other hand, it is known that the warmer areas of the west are good for the production of body fat, and that the colder areas to the east can produce large frames in mature animals.

It is very doubtful whether breed is the main factor in this variation in body size, because it has been found in all the three categories and in all the four grades, as well as in the carcass weights of the Reject cattle.

For the present, one can only theorize and make such suggestions as may later be substantiated by further investigations in the field.

SUMMARY

1. The body size and grading of the wastage cattle have been described. It was found that the average wastage cow is relatively small in size with an average live weight of 1100 lb. or 9¾ cwt. The condition was also disappointingly poor since over one-half of the total wastage cows are in grade C or are ungraded.

2. The seasonality of the sales shows an autumn glut, which appears to increase as the grade or condition deteriorates.

3. The annual variations in body size during the 4 years are very slight, which seems to indicate that under present circumstances the region can only maintain cows of this size.

4. It was shown that the region can be subdivided into four parts in which the average body size for each section falls into one of four well-defined ranges of weight.

5. It is surprising to find that the average body size of the cows of the east as well as of the west is significantly greater than that of the central part of the region devoted to intensive dairying. It is suggested that the animals fit themselves into their particular environment, and therefore the data might serve to illustrate animal ecology in this region.

6. Some information has also been obtained on the carcass weights of the ungraded cattle and on the quantity of condemned meat.

This investigation has formed part of the survey of animal health and production in west Wales, and thanks are due to the scientific branch of Messrs Cooper, McDougall and Robertson, Berkhamsted, for financial support. Grateful acknowledgement is made also to the Ministry of Food (Meat and Livestock Branch) and to Major John Francis, F.S.I., Carmarthen, for permission to examine the records; to Mr Percy George for help with the statistics; to Messrs R. Jones, Ll. R. Evans and R. D. Williams for their work in collating the material.

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433. THE DESIGN AND USE OF A MILKING MACHINE FOR SOWS

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(With 1 Plate and 2 Text-figures)

In the course of an investigation on the yield and composition of sows' milk, we have experienced difficulties in obtaining representative samples of milk for analysis.

The technique adopted was that described by Braude *et al.* (1) in which 5 units of pitocin were injected into the ear vein of the sow and the milk withdrawn by hand. Since the yield of these sows was being measured over a 24 hr. period each week, it was possible to compare the quantities obtained, following injection, with the average amount suckled by the litters during natural sucklings. The comparison was never particularly accurate as we found that two operators, each milking one teat, were all that could be conveniently accommodated on each side of the sow. This meant that only four glands could be milked properly. We found, too, that variations in teat size and mammary gland size affected the speed of withdrawal and the final amount obtained.

The fact that the teats of these sows had two ducts, and the ends of the teats were more or less distorted by frequent suckling, made it extremely difficult to direct all the milk into the containers and quite substantial spillage resulted.

Despite these limitations it was not unusual to find that the yields following injection were in excess of the amounts suckled by the litter, and analyses for fat showed wide fluctuations from week to week.

It was decided that some method of standardizing the actual milking process and ensuring the withdrawal of milk from all functional glands at the same time would need to be evolved before accurate sampling could be carried out. For this reason a milking machine for sows was designed. By using this machine the pitocin injection has been reduced to between 1·5 and 2 units, and the yields obtained at a milking are comparable with the average quantity withdrawn by the litters during the natural suckling process. Details of these results will be published in a subsequent paper.

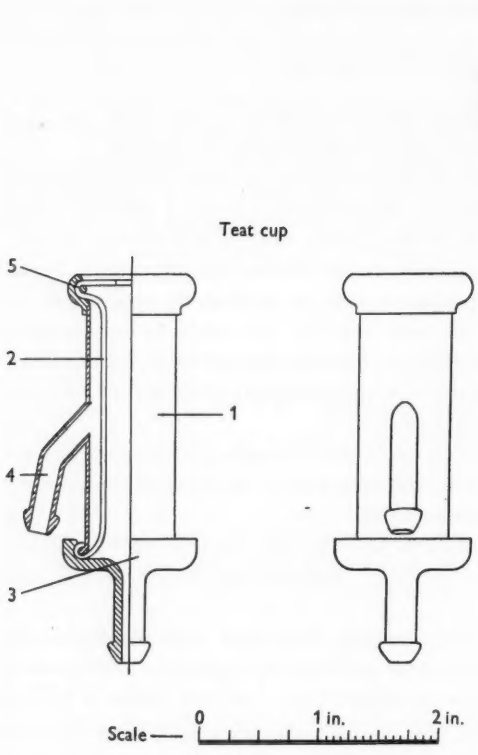
THE MECHANICAL MILKER

The machine used for the pig-milking experiments is based on the principles employed in the conventional cow milker. A vacuum of 14–15 in. of mercury is used to withdraw the milk, while massage and stimulation of the teats are effected by using a stretched liner type of teat cup with pulsating vacuum applied to the outside surface of the liner.

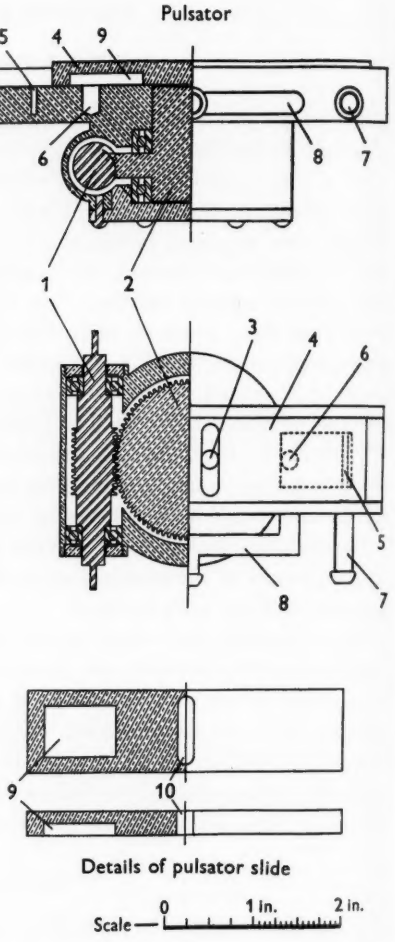
The teat cup used is shown in Text-fig. 1. The dimensions were arrived at by taking a series of measurements of normal sow teats. The cup consists of the main casing (1) containing the rubber liner (2), the lower end of which is folded back over the bottom lip of the main casing and held in place by the cap and nipple (3). The upper end of the liner is stretched over a ring (5) which holds it against the inside of the cup mouthpiece. The

connecting tube (4) applies the pulsator vacuum to the outside of the liner. The nipple on the bottom cap (3) connects the cup to the milk collecting tube.

The pulsator valve is of somewhat novel design in that it is intended to test a possible improvement in the construction of the cow-milker pulsator. The components are shown in Text-fig. 2. The driving worm (1) is connected by a flexible coupling to the shaft of a small high-speed motor. The worm drives the toothed wheel (2) to which is fixed a pin (3),



Text-fig. 1.



Text-fig. 2.

which transmits a reciprocating motion to the slide (4) via a slot in the undersurface of the latter. The outer rectangular ports (5) connect to the pulsator nipples of the teat cups via tubes (7). The circular ports (6) connect by tube (8) to the vacuum source. The slide (4) is a solid rectangle of brass on the lower surface of which are machined the two valve cavities (9) and the central driving slot (10). The pulsator rate used is approximately 100 pulsations per min. The main advantages of the pulsator valve described are: (*a*) the worm

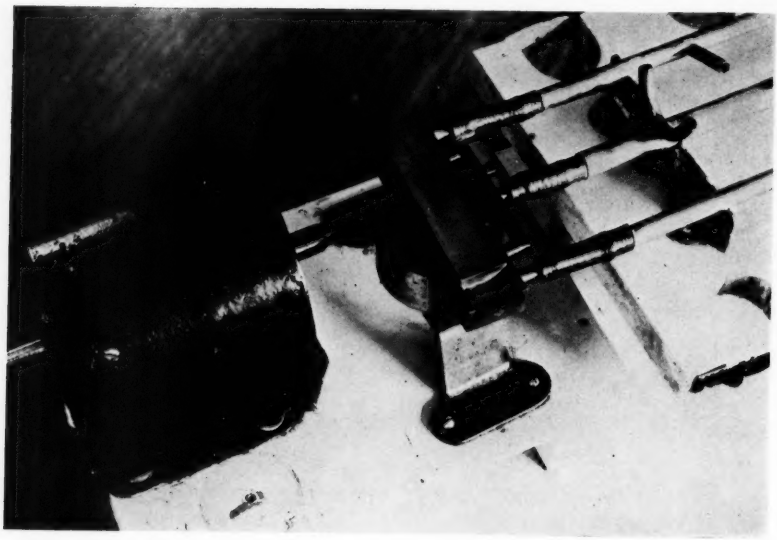


Fig. 1.

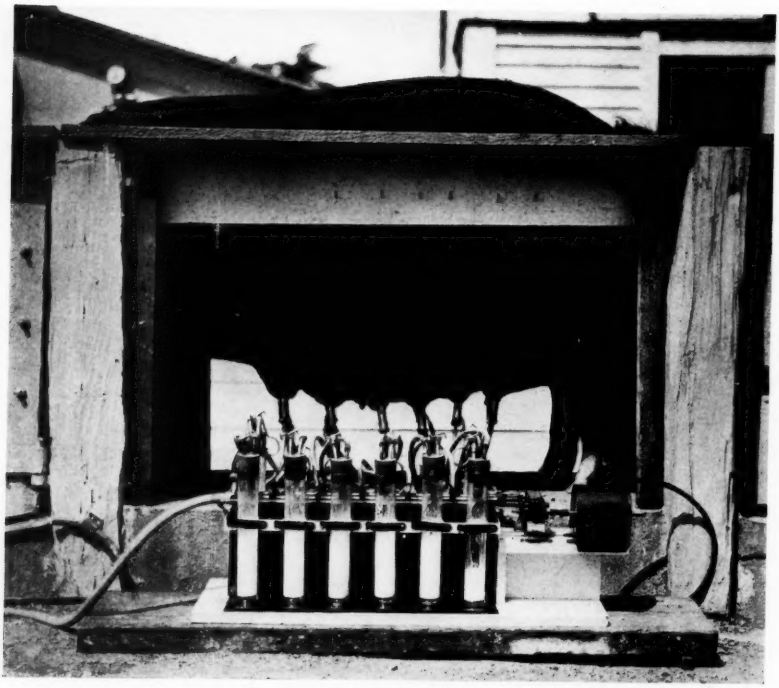


Fig. 2.

drive runs in oil and seepage maintains a film of oil on the surfaces of the slide valve and driving pin; (*b*) all drives are enclosed and so free from dirt; (*c*) there are only three moving parts. The complete pulsator unit is seen in pl. 1, fig. 1. The valve applies pulsation alternately to two sets of six teat cups, the double system being used to minimize vacuum fluctuations in the plant.

The milk from each gland runs to a measuring cylinder so that individual samples are obtained, while the whole system is evacuated by a small standard milking-machine vacuum pump. The general layout of the machine is seen in Pl. 1, fig. 2, which shows the milker in use.

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434. THE BACTERIOLOGICAL QUALITY OF NATIONAL DRIED MILK

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During the last 5 years the Department of the Government Chemist has carried out bacteriological examinations on over 1200 samples of National Dried Milk. These samples came from eleven factories (and repacking depots) in England and Northern Ireland run by eight different proprietors. It is hoped that a note of the results obtained may be of interest as indicating the general level of bacteriological quality recently obtained by British manufacturers of roller-dried full-cream and half-cream milk.

PREVIOUS WORK

L. A. Allen (1) in *Bulletin of the Hannah Dairy Research Institute*, no. 3, 'The properties of milk in relation to the condensing and drying of whole milk, separated milk and whey', gives a summary of papers published on the bacteriology of roller-dried milk up to 1932. Since then the only important paper on this subject is one by C. Higginbottom (2) published in 1944.

FACTORS AFFECTING RESULTS

In considering the figures given in this paper and comparing them with those in the papers quoted, it is desirable to bear in mind two factors which may considerably affect the result, namely: (*a*) method of reconstitution, and (*b*) time of storage before testing.

(*a*) Higginbottom (2) followed the method of reconstitution previously used at the Hannah Institute by Nichols (3), in which an approximately 10% dispersion is made by shaking in cold sterilized water containing some glass beads. The American Dry Milk Institute (4) prescribes reconstitution by shaking in cold water but permits the use of water at 40° C. 'if difficulty is experienced in obtaining satisfactory homogeneous dispersion'. It stipulates, however, that the test portion should not be in contact with water at a temperature above 37° C. for more than 15 min. Higginbottom (5), in a later paper, compares two methods of reconstitution in which a 10% dispersion was made in Ringer's solution (i) at 16–20° C. and (ii) at 50° C., followed by holding at this temperature for 15 min. She claims that the higher temperature gives clearer plates, better replicates and counts which for roller-dried powder are about 40% higher. Hiscox (6) shows that the temperature of reconstitution has a marked influence on the count obtained, the figure for 50° C. being higher than that for room temperature. She recommends reconstitution at 50° C. in Ringer's solution.

(*b*) The effect of storage after manufacture on the bacterial count is considered by Allen (1), who quotes figures showing a rapid fall with time. Nichols (3) for spray powder found a reduction in the count of 67% in 3 months and 73% in 6 months. Higginbottom (2) for roller powder found that a fall in the count usually occurred on keeping but that this was not invariably the case. On the average, she found a decrease in the count of 32% in 3 months, 64% in 6 months and 80% in 9 months.

The roller-dried full-cream or half-cream milk is packed immediately in bulk metal containers, usually holding about 56 lb. The powder is stored for a period not exceeding 6 months but usually considerably less, before being repacked into 20 oz. units in paper bags enclosed in National Dried Milk tins which are given a maximum shelf-life of 6 months from the time of packing, although the powder is usually distributed to the consumer within 2–3 months. The powder may be expected to come off the rollers with a comparatively low count at least as regards mesophilic bacteria (1) and (2). Some bacterial contamination is likely to occur in the first packing into large tins. During storage in these large tins the count is likely to fall and this fall will probably be followed by some additional contamination during the repacking operation. Finally the count may be expected to fall during storage in the small tins. The samples received at the Department of the Government Chemist were unopened 20 oz. tins from the stock actually in course of distribution from local food offices and thus represented National Dried Milk in the condition in which it would reach the recipients.

EXPERIMENTAL TECHNIQUE

For reconstitution a 6 oz. wide-necked sterilized stoppered glass bottle was counterpoised on a balance. The sample was opened and the superficial layer was removed by means of a sterilized metal spatula which was then used to weigh 10 g. of powder into the bottle. Sterilized quarter-strength Ringer's solution at 50° C. was added to the bottle up to a line previously marked at 100 ml., and the bottle was at once stoppered and shaken with five rapid excursions of about 1 ft. The bottle was immediately placed in a bath at 50° C.; kept there for 10 min.; shaken again 40 times and returned to the bath for a further 10 min. The liquid prepared in this way was the 10% solution referred to later and from it a 1% solution was made up in the usual way. On these solutions plate counts were carried out and tests were applied for coliform organisms and for *Clostridium welchii* (7).

For plating, 1 ml. quantities of the 1% solution were added to milk agar in duplicate and incubated at 37° C. for 3 days and at 55° C. for 2 days. The plates were counted in a Mattick counting box using a hand-lens.

For the presumptive coliform test three portions, each 10 ml., of the 10% solution were used. Since August 1947, when a positive result has been obtained, tests have been applied to type the organism present according to the Ministry of Health classification (8).

For the recognition of *Cl. welchii* three portions of 10 ml. each of the 10% solution were placed in test-tubes which had been sterilized after addition of a little petroleum jelly. The tubes were placed in a bath at 80° C. for 20 min. to destroy all vegetative forms and then incubated at 37° C. for 3 days. A positive result was recorded when the milk clotted and the petroleum seal was driven up the tube by the evolved gas.

The results obtained were studied by the Statistics and Intelligence Division of the Ministry of Food by means of the Hollerith machine. The information supplied by us with reference to each sample was as follows:

(1) Year of receipt.
(2) Distinguishing number.
(3) A code number representing the source of the sample.
(4) Approximate age since repacking into 20 oz. tins.
(5) Bacterial count at 37° C.

(6) Bacterial count at 55° C.
(7) Logarithm of count at 37° C.
(8) Logarithm of count at 55° C.
(9) Result of Coli test.
(10) Result of Welchii test.

Notes on above

(3) By 'source' is meant the depot at which the powder was repacked into 20 oz. tins. The sources were of three kinds:

(*a*) Factories repacking powder of their own manufacture only. These are seven in all, viz. nos. 1, 2, 3, 6, 10, 11 and 12.

(*b*) Factories repacking powder made by other makers as well as their own product—four in all, viz. nos. 4, 5, 9 and 13.

(*c*) Sources which are repacking depots only and do not manufacture—three in all, viz. nos. 7, 8 and 14.

In some cases no information as to the source was available and to cover these a composite group was included for which the source was unknown.

(4) Age. We had no means of determining the period for which the powder had been stored in the 56 lb. tins. The period since repacking into 20 oz. tins could only be determined approximately. Perhaps for these reasons it will be seen later that no correlation between age and count was found. In some cases no information at all about age was available. Such samples were allotted a purely fictitious 'age' of 9 months. This figure is not an estimate of age but purely an indication to allow these samples to be sorted out by the machine.

(5)–(8). In accordance with the usual practice in treating bacteriological results statistically, logarithms of counts were considered as well as the arithmetical values. See Robertson & Frayer (9) and Mattick, McClemont & Irwin (10).

The results of the analysis of the figures are given in Tables 1–7.

DISCUSSION OF RESULTS

Table 1

The average level of bacterial contamination for both mesophilic and thermophilic bacteria is comparatively low. The distributions of the figures about the means—very skew for the arithmetic means, normal for the geometric means—was held by the statisticians to be ample justification for using the logarithmic figures only in their subsequent discussions.

Table 1. *Arithmetical and geometrical mean counts at* 37 *and* 55° C.

	Plate count at 37° C.	Plate count at 55° C.
Number of milk samples ...	1288	1150
Arithmetic mean count	2810	3870
Percentage of samples above	22·4	21·1
Percentage of samples below	77·6	78·9
Geometric Mean count*	1160	1070
Percentage of samples above	50·2	50·7
Percentage of samples below	49·8	49·3

* The geometric mean is the antilogarithm of the mean log count.

Table 2

Higginbottom's figures are shown for comparison with ours. The similarity is striking. Her mean log mesophilic count is almost the same as ours, with a slightly higher standard deviation. Her thermophilic count is slightly lower than ours with almost identical standard deviation.

Table 2. *Logarithmic parameters of the distribution*

	Plate count at 37° C.	Plate count at 55° C.
Number of milk samples ...	1288	1150
Mean log	3·06 (2·90)*	3·03 (2·36)*
Log standard deviation	0·564 (0·81)	0·700 (0·72)
Coefficient of variation (%)	18·4	23·1
Standard error as percentage of mean log	0·5	0·7

* Higginbottom's figures are shown in brackets

Tables 3 *and* 5. *Trend through time*

No tendency towards regular increase or decrease in counts throughout the period of 6 years considered can be observed, either in the combined results of all sources (Table 3) or in the results of any individual source (Table 5).

Table 3. *Mean log counts for each year separately*

(Combined results of all sources*)

	37° C.		55° C.	
Year	Mean log count	No. of samples†	Mean log count	No. of samples†
1944	3·14	176	2·93	110
1945	3·02	271	2·97	153
1946	3·07	281	3·00	114
1947	3·04	244	3·17	194
1948	3·08	253	3·14	239
1949	3·00	63	2·66	60

* Excluding those for which the age and source were not known.
† For note on 'sources' see Table 4.

Table 4

(*a*) *Correlation between count and age.* No correlation between count and age was found. In view of the results of other workers already quoted this may appear surprising. It must, however, be remembered that the 'age' figure considered in this work is not the time elapsed since manufacture but only since repacking into 20 oz. tins. The time the powder spent in 56 lb. tins previous to repacking is variable and unknown.

(*b*) *Comparison between samples from different sources.* The table gives the results obtained upon the products of the eight sources which went in forty samples or more and includes also the composite group (no. 15) which contained all samples the origin of which was unknown. The sources appear to fall into two groups at each temperature.

37° C.	Group A	Sources no. 3(*a*), 8(*c*), 13(*b*)	Mean log about 2·8
55° C.	Group A	Sources no. 3(*a*), 5(*b*), 8(*c*), 9(*b*) and 13(*b*)	Mean log about 2·7
37° C.	Group B	Sources no. 2(*a*), 4(*b*), 5(*b*), 9(*b*), 10(*a*), 15	Mean log about 3·1
55° C.	Group B	Sources no. 2(*a*), 4(*b*), 10(*a*), 15	Mean log varies from 2·9 to 3·5

Against the number of each source is given its type, (*a*), (*b*) or (*c*). It will be seen that there is no apparent relation between the type of the source and the group into which it falls.

Table 4. *Comparison of samples from different sources,* and correlation between count and age of sample*

(Log plate counts to base 10)

Source no.	Source type	37° C. No. of samples	Mean count (log)	Standard deviation (log)	Percentage coefficient of variation	Standard error as percentage of mean	Mean age (mos.)	Correlation age × count	55° C. No. of counts	Mean count (log)	Standard deviation (log)	Percentage coefficient of variation	Standard error as percentage of mean	Mean age (mos.)	Correlation age × count
02	*a*	132	3·12	0·464	14·8	1·3	3·6	−0·105	117	3·41	0·686	19·5	1·8	3·6	—
03	*a*	155	2·79	0·494	17·7	1·4	3·5	−0·062	143	2·79	0·680	24·4	2·0	3·3	—
04	*b*	149	3·14	0·583	18·6	1·5	3·9	−0·022	138	3·21	0·602	18·7	1·6	3·8	—
05	*b*	71	3·15	0·533	16·9	2·0	3·6	−0·073	60	2·68	0·551	20·6	2·7	3·6	—
08	*c*	71	2·82	0·434	15·4	1·8	2·8	+0·062	70	2·64	0·665	25·2	3·0	2·8	—
09	*b*	52	3·14	0·374	11·9	1·7	4·4	+0·016	47	2·87	0·474	16·5	2·4	4·3	—
10	*a*	237	3·09	0·506	15·8	1·0	3·9	−0·118	208	3·25	0·663	20·4	1·4	3·7	—
13	*b*	40	2·89	0·573	19·8	3·1	3·3	−0·131	40	2·67	0·612	23·0	3·7	3·3	—
15 (unknown)		330	3·13	0·613	19·6	1·1	Age unknown		280	2·98	0·676	24·3	1·5	Age unknown	
All		1288	3·06	0·564	18·4	0·5	—	—	1150	3·03	0·700	23·1	0·7	—	—

* Sources include factories and repacking depots. They are divided into 'Types' as follows:

Type (*a*). Factories repacking powder of their own manufacture only.

Type (*b*). Factories repacking powder made by other makers as well as their own product.

Type (*c*) Sources which are repacking depots only and do not manufacture.

Table 5. *Bacterial plate counts: mean log values shown within sources and years*

Source no.	Source type	1944	1945	1946	1947	1948	1949
2	*a*	—	3·04	3·16	3·03	3·40	—
		(5)	(33)	(38)	(36)	(17)	(3)
3	*a*	2·73	2·76	2·72	2·61	3·03	—
		(18)	(30)	(23)	(28)	(48)	(8)
4	*b*	3·48	2·83	3·14	3·07	3·10	3·29
		(20)	(21)	(17)	(18)	(52)	(19)
5	*b*	3·18	—	3·24	—	3·01	—
		(21)	(8)	(21)	(9)	(10)	(2)
8	*c*	—	—	—	2·82	2·77	—
			(4)	(6)	(31)	(28)	(2)
9	*b*	3·07	—	—	—	—	—
		(25)	(7)	(9)	(7)	(7)	—
10	*a*	3·42	3·15	—	3·16	3·24	3·12
		(20)	(48)	(6)	(51)	(44)	(13)
15		3·39	3·05	3·00	3·31	3·16	—
		(51)	(49)	(93)	(50)	(14)	(3)
All sources		(176)	(271)	(181)	(244)	(253)	(63)

Notes: (*a*) Numbers of observations in brackets. (*b*) Averages based on less than ten counts have been omitted.

Table 6. *Correlation between plate counts at* 37 *and* 55° C. *for samples from various sources*

Source no.	Source type	Number of pairs of observations, i.e. plates counted at both temperatures	Correlation coefficient 37° × 55° C.
02	*a*	116	+0·322
03	*a*	142	+0·571
04	*b*	137	+0·238
05	*b*	60	+0·266
08	*c*	70	+0·300
09	*b*	47	−0·038
10	*a*	208	+0·133
13	*b*	40	+0·588
15		276	+0·255
All sources		1143	+0·3001

Table 7. *Results of Coli and Welchii tests for samples from different sources*

		Coli test			Welchii test	
Source no.	Source type	Percentage present	S.E.	No. of tests	Percentage present	S.E.
02	*a*	8·27	±2·39	133	20·30	±3·49
03	*a*	3·85	±1·54	156	8·33	±2·21
04	*b*	8·67	±2·30	150	15·33	±2·94
05	*b*	1·41	±1·40	71	11·27	±3·75
08	*c*	1·41	±1·40	71	9·86	±3·54
09	*b*	1·92	±1·90	52	11·54	±4·43
10	*a*	4·22	±1·30	237	18·14	±2·50
13	*b*	2·50	±2·47	40	7·50	±4·16
15		7·44	±1·43	336	16·67	±2·03
All sources		5·40	±0·62	1297	14·34	±0·97

Coli and Clostridium welchii

Table 7 sets out the results of the Coli and Welchii tests for each source separately. The standard error of the proportion reacting is given in each case. It will be noticed that sources 3, 8 and 13 have the lowest proportions reacting to the Welchii tests and sources 5 and 9 show a lower than average proportion. The spores of *Cl. welchii* can survive the

drying process, hence the results of the Welchii tests are related to the quality of the milk reaching a factory.

With regard to the Coli tests the same five sources show the smallest proportion of samples reacting.

It will be observed that only one of these five sources is of type (*a*), viz. a factory repacking only its own product. The other four sources all deal with powder of varied origin.

The results of typing tests which have, since August 1947, been applied to all samples giving a positive reaction to the presumptive Coli test have been as follows:

Total positive presumptive Coli results	19
Aerogenes Type I	17
Aerogenes Type II	1
Intermediate	1

Correlation between counts at 37° *and* 55° C. Table 6 gives the correlation between counts at 37 and 55° C. for the majority of sources. The overall correlation, that is the correlation figure for all sources, is 0·300. This, though low, is not insignificant. For different sources it varies between a minimum of −0·038 and a maximum of +0·588. These facts receive an explanation if the existence of two modes of contamination is assumed in one of which, (*a*), correlation exists between the numbers of the two kinds of bacteria introduced, whilst in the other, (*b*), the numbers vary quite independently. Two channels of contamination have been postulated earlier in this paper. Perhaps we may see in the repacking operations correlated source of contamination with both types of bacteria corresponding to (*a*), and in the bacteria surviving drying a source in which thermophilics can vary independently of mesophilics corresponding to (*b*). Thermophilic bacteria are more likely to survive the drying process than mesophilics. They can multiply rapidly in hot milk and given suitable conditions such as might exist in an infected preheater the number present in the milk reaching the rollers might be large. A proportion of these would normally be found alive in the dried product.

From the data available it is found that, as compared with the others, two factories repacking only the product of their own manufacture had a significantly higher proportion of cases in which the thermophilic count was more than three times the mesophilic count. In these factories pre-drying multiplication of thermophilics is indicated. From one of these factories samples were received over a short period which showed really high figures for thermophilics, reaching a maximum figure of 160,000 per g. of powder.

Standards of quality

Higginbottom (2) considers the possibility and desirability of setting up a bacterial standard for roller-dried milks in this country similar to that of the American Dried Milk Institute (4). She thinks action on these lines may be difficult in view of the effect on the count of the age of the sample and the manner of testing, but expresses the opinion that a maximum count of 5000 per g. 'would... not appear to be unduly rigorous for a first grade product'.

Higginbottom's suggestion may be compared with existing American and Dutch standards which are as follows:

Standard of American Dry Milk Institute (4) *for full-cream roller powder*

Grade	Bacterial count per g. powder
Extra	Less than 50,000
Standard	Less than 125,000

Dutch Standard (Zuivel Kwaliteitscontrôle Bureau Regulations for Examination and Grading of Dried Milk)

Grade	Bacterial count per g. powder
Extra	Less than 150,000
Standard	Less than 500,000
Second	Less than 1,000,000

The figures given in Table 4 should be useful in any further consideration of this question. They enable one to calculate the proportion of samples of National Dried Milk which, during the period covered, have actually been found to comply with any possible standard. Such calculations may relate (*a*) to the source with the best record and so point to an optimum standard, or (*b*) be based upon results obtained from samples from all sources and so indicate a reasonable 'current' standard.

From a knowledge of the logarithmic mean count 'LMC' and of the standard deviation 'S' we can write down the approximate proportion of samples giving counts within any stated limits. A few examples are given in the following table:

Limits	Percentage of samples exceeding the limit
LMC + 3S	Negligible
LMC + 2S	Not more than 2·5
LMC + 1S	Not more than 16

In Table 8 these principles are applied (*a*) to the results from source 8, and (*b*) to the results from all sources. Unfortunately, source 8 is a source of Type (*c*), that is a repacking depot only. Admittedly a source of Type (*a*)—a factory repacking only its own product—would afford more convincing figures for setting up optimum standards. Yet as a channel through which powder of a particularly high grade has actually flowed source 8 has its value for indicating a possible level of achievement.

Table 8. *Maximum logarithmic count*

Percentage of samples exceeding limit	Optimum (*a*)		Current (*b*)	
	37° C.	55° C.	37° C.	55° C.
Negligible	4·122	4·635	4·752	5·130
2·5	3·688	3·970	4·188	4·430
16	3·254	3·305	3·624	3·730

In Table 9 the antilogarithms of the maximum logarithmic count have been taken and the table expresses the results as arithmetical counts.

Table 9. *Maximum arithmetical counts*

Percentage of samples exceeding limit	Optimum		Current	
	37° C.	55° C.	37° C.	55° C.
Negligible	13,200	43,200	56,500	135,000
2·5	4,900	9,300	15,400	26,900
16	1,800	2,000	4,210	5,370

It will be seen that almost the whole production reached the American standard for extra grade (count at 37° C. less than 50,000) and more than 84% reached the standard of not more than 5000 per g. suggested by Higginbottom. The results from source 8 show that a standard as stringent as 2000 per g. for both mesophilic and thermophilic organisms can be reached in powder from a selected source with respect to 84% of its product.

As an alternative to a standard setting limits separately for mesophils (M) and thermophils (T), it might sometimes be useful to consider an index of quality which depended upon both these counts. This is obtained by combining the two counts in such a way that the resultant has the minimum coefficient of variation. Calculated by an adaptation of the technique of Steiner (11) from the data given in Table 4, the 'optimum combination' would take the approximate form $\log M\sqrt{T}$. For source 8, the best source considered, less than 1 sample in 10 would give $\log M\sqrt{T}$ more than 5. If M and T are approximately equal the value 5 for the optimum combination corresponds to a value of 2155 for either count.

SUMMARY

This paper presents in statistical form the results of the bacteriological examination of over 1200 samples of National Dried Milk during the period 1944–9. Factors which may affect the results are discussed. The general level of bacterial quality disclosed is high, but appreciable differences are shown in the standards reached at different factories and repacking depots. No general trend with time was observed in the figures throughout the period of 6 years considered, and no correlation was observed between the counts obtained and the time which had elapsed since packing into 20 oz. tins. Between counts at 37 and at 55° C. some correlation was noted in the products of most factories and depots, and the significance of this observation is discussed. The counts obtained are compared with various standards which have been put forward or are now in operation, and ways are suggested in which the figures might be used to set up standards of bacterial quality for roller-dried milk powder in this country.

We wish to record our obligation to the staff of the Statistics and Intelligence Branch of the Ministry of Food by whom the statistical analysis of the figures was carried out and to thank the Government Chemist and the Ministry of Food for permission to publish this paper.

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435. KEEPING QUALITY AND RAW-MILK GRADING

II. THE EFFECT OF STORAGE ON THE RESULTS OF KEEPING QUALITY AND DYE TESTS

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(With 4 Figures)

CONTENTS

	PAGE
Introduction	43
Experimental	44
Source and treatment of samples	44
Experiment 1	45
Experiment 2	46
Experiment 3	46
Experiment 4	48
Results and discussion	49
Methods of statistical analysis	49
Consideration of figures	56
The regression coefficient B	57
The correlation coefficient r	60
The rate of deterioration of milk during pre-test storage	64
A general formula for the rate of deterioration	65
Comparison of calculated with observed results	67
A possible grading scheme for milk	68
Relationship between keeping qualities at different incubation temperatures	69
Summary and conclusions	70
References	71

INTRODUCTION

The time-temperature conditions under which milk samples are kept before examination have a profound effect upon the results of all bacteriological tests. In Britain, it is customary to store samples for a prescribed period before testing is begun (1, 2). It has not been considered practicable to control the temperature of routine samples throughout the period of pre-test storage, because this would involve the sampling of all milk at the time and place of production, and the use of elaborate equipment to maintain the desired temperature. In the application of routine tests, some attempt is made to offset the effect of uncontrolled pre-test storage conditions. Thus, when testing designated milks (1) the methylene-blue standard is varied according to the season; in summer, samples not

reducing methylene blue in 4½ hr. are classed as satisfactory, but in winter the standard is 5½ hr. With the routine resazurin test (2), the time of incubation of samples at 37·5° C., after the addition of resazurin, is varied according to the mean shade atmospheric temperature during the 24–28 hr. pre-test storage period.

Despite the adoption of a fixed period of storage before testing, and the adjustments in standards designed to offset the effect of temperature during that period, wide fluctuations occur in the proportion of samples failing at different seasons of the year. Nichols & Edwards (3), Thomas, Bowie, Peregrine & Phillips (4), Mitchell (5), and Savage (6) in Britain, and Smythe (7) in Australia have given figures which emphasize the dependence of results on the temperature of storage of the milk before testing begins.

A series of experiments was planned to obtain information about the effect of time and temperature of pre-test storage on test results, and, in particular, about the possibility of applying a correction factor to the results which would eliminate or minimize the effects of variations of pre-test storage conditions in routine work. Apart from the work of Smythe (7), published just after our own experimental work was begun, and of the later work by Morton & Vincent (8, 9), we are not aware of any other data of a similar character. Smythe's data cover storage at temperatures in the range 5–35° C. for periods up to 5 hr. only; Morton & Vincent's storage for periods of 4–24 hr. at temperatures of 0–30° C.

EXPERIMENTAL

Four separate experiments were planned. As in the experiments described in Part I (10), the experimental work was carried out at the three centres *K*, *L* and *M*.

Source and treatment of samples

The samples were taken at farms accessible to the testing laboratories. To include milks of a wide range of bacteriological quality, the farms were selected according to their routine resazurin test results during the previous 6–12 months. In any one experiment no farm was sampled more than once, except in the first experiment when a few of the farms sampled during the winter period were again sampled during the summer period.

Broadly the plan in each of the experiments was to bring samples to the laboratory with the minimum of delay after completion of milking on the farms, and then to divide them into subsamples for storage in thermostatically controlled water-baths at different temperatures for prescribed periods of time before testing. For convenience, morning milk only was sampled.

Milking at the farms generally finished between 7 and 8 a.m., and the samples reached the laboratories between 8.30 and 9.30 a.m. No special precautions were taken to control the temperature of the samples during transit to the laboratories. No undue rise in temperature was likely during transit: moreover, the bacteriostatic properties of the milk would be operative during this period.

On arrival in the laboratory each sample was thoroughly mixed and the necessary number of subsamples prepared. At 11 a.m. (10 a.m. in Exps. 3 and 4) all the subsamples were transferred to thermostatically controlled water-baths for storage at temperatures ranging from 2–24° C.; these temperatures cover the range normally met in Britain. An additional subsample was stored at shade atmospheric temperature, the normal routine method of storage (1, 2). In general, the temperature of the water-baths, recorded at

3 or 4 hr. intervals during the storage period, never varied by more than ±0·5° C. of the stated temperature, and the mean of the readings never varied by more than ±0·1° C. For storage at 2° C., however, a water-bath placed in a refrigerator was used and some difficulty was experienced in maintaining this temperature. There were appreciable fluctuations in individual readings, and the mean temperature of storage was generally higher than 2° C. The overall mean temperature for samples in the first experiment was 3·6 instead of 2° C. and in the second experiment 2·7° C. In the subsequent statistical analysis of the results, therefore, the temperature of storage was taken as 4° C. in the first and 3° C. in the second experiment. In the third and fourth experiments storage at 2° C. was discontinued and a temperature of 4° C. substituted; no difficulty was experienced in maintaining the latter temperature.

In these experiments the testing procedures detailed in Part I (10) were followed throughout.

Experiment 1

This experiment extended from 5 November 1945 to 24 June 1946, and therefore included both winter and summer conditions.

Each sample consisted of approximately 2 pints, from which eight subsamples of approximately 120 ml. were prepared. Starting at 11 a.m. on the day of sampling, one of the subsamples was stored for 22 hr. at each of the temperatures 2, 10, 14, 18 and 22° C., and shade atmospheric temperature; the remaining two subsamples were stored for 26 hr., one at 14° C. and the other at 22° C. At the end of the storage periods (9 a.m. on the day following sampling for the six subsamples stored for 22 hr., and 1 p.m. for the two stored for 26 hr.) the subsamples were incubated at 18° C., and their keeping quality determined using precipitation with 68% alcohol (A.P.T.) as the end-point with observations at 4 hr. intervals (10). At the time of transfer to the 18° C. bath for incubation after 22 hr. storage, 10 ml. was withdrawn from each subsample for the determination of the methylene-blue reduction time at 37·5° C.

Plan of Experiment 1

Time	Storage period (hr.)	Treatment
Day of sampling:		
7.30–8.30 a.m.	—	2-pint sample taken at farm
8.30–9.30 a.m.	—	Sample reached laboratory
9.30–11 a.m.	—	Preparation of eight subsamples (120 ml. each)
11 a.m.	0	Transfer of eight subsamples to storage baths
		2° C. 10° C. 14° C. —— 14° C. 18° C. 22° C. —— 22° C. Shade atmospheric temperature
Day after sampling:		
9 a.m.	22	Six subsamples transferred to 18° C. incubation bath for keeping quality determination: test with 68% alcohol at 4 hr. intervals to end-point Methylene-blue test at 37·5° C.
1 p.m.	26	Two subsamples (stored at 14 and 22° C.) transferred to 18° C. incubation bath and tests with 68% alcohol carried out as at 9 a.m.

This experiment provided information on the effect of different temperatures of storage but only a limited amount of information on the effect of duration of storage. Accordingly, a second experiment was planned in which subsamples maintained at the same temperatures as in the first experiment were stored for 9, 21 and 30 hr. before examination.

Experiment 2

This experiment was carried out during July and August 1946. For reasons which will emerge later (p. 57), examination of samples by the methylene-blue test was discontinued. Keeping quality tests only were applied.

Three sets of six subsamples, each approximately 60 ml., were prepared from an original bulk of approximately 2 pints. As in Exp. 1, the subsamples were placed in the constant temperature water-baths for storage at 11 a.m. on the day of sampling. Storage was at 2, 10, 14, 18 and 22° C., and shade atmospheric temperature for periods of 9, 21 and 30 hr. At the end of the appropriate storage period the subsamples in each set were incubated at 22° C. (instead of 18° C. as in Exp. 1), and their keeping quality determined. Three measures of keeping quality—precipitation with 68% alcohol (A.P.T.), 50% alcohol, and clot-on-boiling (C.O.B.)—were used with observations at 3 hr. intervals. No other tests were applied to the subsamples.

Plan of Experiment 2

Time	Storage period (hr.)	Treatment
Day of sampling:		
7.30–8.30 a.m.	—	2-pint sample taken at farm
8.30–9.30 a.m.	—	Sample reached laboratory
9.30–11 a.m.	—	Preparation of subsamples: three sets (nos. 1–3) of 6/60 ml. quantities for keeping quality tests
11 a.m.	0	Transfer of three sets (nos. 1–3) of subsamples to storage baths Set 1 Set 2 Set 3 2° C. 10° C. 14° C. 18° C. 22° C. Shade atmospheric temperature
8 p.m.	9	Set 1 transferred to 22° C. incubation bath for keeping quality determinations: tested with 68% alcohol (A.P.T.), 50% alcohol, and clot-on-boiling (C.O.B.) at 3 hr. intervals to end-point
Day after sampling:		
8 a.m.	21	Set 2 transferred to 22° C. incubation bath and tests carried out as at 8 p.m.
5 p.m.	30	Set 3 transferred to 22° C. incubation bath and tests carried out as at 8 p.m.

Experiment 3

This experiment was carried out during the second half of 1947. It followed closely the general design of the previous experiments. Its purpose was to check the results of the earlier experiments; to obtain information about a wider range of incubation temperatures;

and to explore the possible use of the methylene-blue test with incubation at 18, 22 or 24° C. As in the second experiment, samples were not examined by the methylene-blue test at 37·5° C.

The experiments in Part I(10) had shown that there was a close relationship between keeping quality determinations measured by A.P.T. and C.O.B.: in the third experiment, because of the large number of subsamples to be tested at each observation, C.O.B. only was done.

At the time of planning this experiment the analysis of the results of the first two experiments had progressed to a stage which indicated the possibility of a routine grading scheme based on the measurement of keeping quality with adjustments in the time of testing to compensate for variations in temperature during pre-test storage: further, that incubation of samples at a higher temperature than 22° C. might prove advantageous for routine application. Accordingly, incubation at 24° C. was included in the third experiment.

Methylene-blue reduction times with incubation at 18, 22 and 24° C. were determined, since no information was available from the earlier experiments about the effect of time and temperature during pre-test storage on methylene-blue results with incubation at temperatures other than 37·5° C. Some of the methylene-blue tests were done in duplicate, one of the tubes being inverted at each observation, the other remaining undisturbed until the end-point was reached. At the lower incubation temperatures—18, 22 or 24° C.—it is generally necessary to incubate the methylene-blue tubes for long periods which include the night interval, as, for example, in the methylene-blue test used for heat-treated milk in Scotland(11). This precludes frequent inversion of tubes. Nevertheless, in view of the known importance of inversion of tubes in dye tests at 37·5° C.(12) a comparison of results with and without inversion was thought desirable.

In Exp. 3 all the subsamples were prepared from an original bulk sample of approximately 7 pints. The subsamples for the keeping quality test (C.O.B.) and for the methylene-blue test were transferred to their respective storage baths at 10 a.m. (instead of 11 a.m. as in Exps. 1 and 2) on the day of milking.

Subsamples for the keeping quality (C.O.B.) *tests.* As in Exp. 2, each subsample for the C.O.B. test was approximately 60 ml. A total of fifty-four subsamples was prepared and divided into nine sets with six subsamples in each set. One subsample from each of the nine sets was stored at each of the temperatures 4, 10, 14, 18, 22 and 24° C. Three sets were stored at these temperatures for 7 hr.; one set was then incubated at 18° C., another set at 22° C., and the third set at 24° C. A further three sets were stored for 17 hr., and the last three sets for 23 hr. respectively, after which incubation was at 18, 22 and 24° C. as detailed above for the first three sets. At 3 hr. intervals after transfer to the incubation baths the C.O.B. test was applied to each subsample until a positive result was obtained.

Subsamples for the methylene-blue tests. For the methylene-blue test 10 ml. of milk and 1 ml. of methylene-blue in $6 \times \frac{5}{8}$ in. tubes closed with rubber bungs were used. Methylene blue was added to all the tubes at 5 p.m. on the day the milk was produced and the tubes inverted twice.

A total of seventy-two tubes were set up: fifty-four received exactly the same storage and incubation treatments as detailed above for the C.O.B. test. These tubes were observed for reduction of the dye and inverted twice at 3 hr. intervals from the time of transfer to the 18, 22 and 24° C. incubation baths. The remaining eighteen tubes were stored at 4, 10,

14, 18, 22 and 24° C. for 7 hr., and subsequently incubated at either 18, 22 or 24° C. until reduced. These latter tubes were inverted when the dye was added but not at subsequent observations.

Plan of Experiment 3

Time	Storage period (hr.)	Treatment
Day of sampling:		
7.30–8.30 a.m.	—	7-pint sample taken at farm
8.30–9 a.m.	—	Sample reached laboratory
9–10 a.m.	—	Preparation of subsamples: nine sets (nos. 1–9) of 6/60 ml. quantities for keeping quality tests using C.O.B. as end-point, and twelve sets of 6/10 ml. amounts for the methylene-blue test
10 a.m.	0	Subsamples transferred to storage baths C.O.B. sets 1–9 (54/60 ml. quantities) / Methylene-blue sets 1–12 (72/10 ml. tubes) 4° C. 10° C. 14° C. 18° C. 22° C. 24° C.
5 p.m.	7	C.O.B. Set 1 transferred to 18° C. incubation bath, set 2 to 22° C. bath, set 3 to 24° C. bath; C.O.B. test applied at 3 hr. intervals to end-point *Methylene blue.* Dye added to all seventy-two tubes. Sets 1–3 and 10–12 to 18, 22 and 24° C. incubation baths respectively Sets 1–3 observed and inverted at 3 hr. intervals to end-point Sets 10–12 observed at 3 hr. intervals but not inverted
Day after sampling:		
3 a.m.	17	C.O.B. Sets 4–6 transferred to 18, 22 and 24° C. incubation baths respectively; C.O.B. test applied at 3 hr. intervals to end-point *Methylene-blue.* Sets 4–6 transferred to 18, 22 and 24° C. incubation baths; tubes observed and inverted at 3 hr. intervals to end-point
9 a.m.	23	C.O.B. Sets 7–9 transferred to 18, 22 and 24° C. incubation baths respectively; C.O.B. test applied at 3 hr. intervals to end-point *Methylene blue.* Sets 7–9 transferred to 18, 22 and 24° C. incubation baths respectively; tubes observed and inverted at 3 hr. intervals to end-point

Experiment 4

This experiment was carried out during June and July 1949. Its purpose was to determine the effect of a short period of storage on the results of the methylene-blue test at 37·5° C.

The plan of the experiment was essentially the same as for Exp. 1, but with storage for 8 hr. instead of 22 hr. From each sample of morning milk five subsamples of approximately 60 ml. were prepared. Between 10 a.m. and 6 p.m. on the day of sampling one of the subsamples was stored at each of the temperatures 4, 10, 14, 18 and 22° C. Between 5.30 and 6 p.m. duplicate 10 ml. quantities from each subsample were transferred aseptically into sterile $6 \times \frac{5}{8}$ in. test-tubes closed with a sterile rubber bung for the methylene-blue test. Testing was begun at 6 p.m. on one of the duplicate 10 ml. portions, while the other was placed in a refrigerator until 10 a.m. next morning and then tested.

In addition to the subsamples detailed above, a methylene-blue test on each sample was begun at 10 a.m. on the day of sampling. With all the methylene-blue tests the tubes were examined and inverted every $\frac{1}{2}$ hr. until the dye was reduced (1).

Plan of Experiment 4

Time	Storage period (hr.)	Treatment
Day of sampling:		
7.30–8.30 a.m.	—	¾-pint sample taken at farm
8.30–9.30 a.m.	—	Sample reached laboratory
9.30–10 a.m.	—	Preparation of five subsamples of 60 ml. each, and one subsample of 10 ml.
10 a.m.	0	Transfer five subsamples to storage baths 4° C. 10° C. 14° C. 18° C. 22° C. Methylene-blue test at 37·5° C. begun on 10 ml. subsample. Tube observed and inverted at ½ hr. intervals to end-point
6 p.m.	8	Duplicate 10 ml. amounts in methylene-blue tubes prepared from each of the five subsamples. Sets 1 and 2 Set 1. Methylene-blue test at 37·5° C. begun. Tubes observed and inverted at ½ hr. intervals to end-point Set 2. Transferred to the refrigerator
Day after sampling:		
10 a.m.	8 plus 16 in refrigerator	Set 2. Methylene-blue test at 37·5° C. begun. Tubes observed and inverted at ½ hr. intervals to end-point

RESULTS AND DISCUSSION

In each experiment approximately the same number of samples was examined at each of the three centres *K*, *L*, *M*, and the samples were evenly spread throughout each experimental period. In the first experiment 139 samples were examined, 71 in the second, 66 in the third, and 36 in the fourth.

Throughout these experiments, as in those described in Part I, the results for the different tests were deemed to be the times to end-point *after* transfer to the incubation baths. Not infrequently, some of the subsamples stored at the higher temperatures, e.g. 22° C., had reached the end-point of a particular test at the time of transfer to the incubation baths and could not be used in the analysis. Further samples were omitted because storage temperature limits were exceeded. The number of subsamples in each experiment used in the statistical analysis of the results is given for each centre in Table I, in which is also given all the values of *B* and *r* discussed later in the Report.

Methods of statistical analysis

The first step in the analysis was to consider the curves obtained for each test by plotting, for each sample, the times to end-point after pre-test storage at the various temperatures. The curves obtained with twelve typical samples examined for keeping quality at 18° C. (A.P.T. end-point) in the first experiment are shown in Fig. 1; the curves are representative of the various series of curves for keeping quality (A.P.T. and C.O.B.) throughout the three experiments. Fig 2 illustrates the curves for the same twelve samples after identical storage conditions but tested by methylene blue at 37·5° C., and Fig. 3 shows a selection of curves for the methylene-blue test at 37·5° C. after 8 hr. storage at controlled temperatures (Exp. 4). These curves will be referred to as 'temperature compensation curves'.

Table 1. *Values of the regression coefficients (B) and the correlation coefficients (r) obtained in the experiments on the effect of storage on keeping quality and dye tests*

Storage Temp. (° C.)	Storage Time (hr.)	Incubation temp. (° C.)	No. of samples Centre K	No. of samples Centre L	No. of samples Centre M	Value of regression coefficient (B) Centre K	Value of regression coefficient (B) Centre L	Value of regression coefficient (B) Centre M	Value of correlation coefficient (r) Centre K	Value of correlation coefficient (r) Centre L	Value of correlation coefficient (r) Centre M
Precipitation with 68% alcohol (A.P.T.)											
Exp. 1, Winter											
4	22	18	33	35	20	0·866	0·991	0·852	0·920	0·950	0·903
10	22	18	33	34	20	0·960	0·949	0·955	0·911	0·954	0·940
14	22	18	33	35	20	0·905	0·970	0·922	0·927	0·969	0·951
14	26	18	28	35	20	0·852	0·934	0·865	0·881	0·952	0·973
18	22	18	33	35	20	1·000	1·000	1·000	1·000	1·000	1·000
22	22	18	26	22	13	0·889	1·064	0·966	0·833	0·880	0·932
22	26	18	13	16	10	0·751	1·373	1·204	0·467	0·754	0·925
Exp. 1, Summer											
4	22	18	9	12	28	1·109	1·078	1·106	0·961	0·967	0·892
10	22	18	9	12	28	0·910	1·152	1·199	0·973	0·960	0·870
14	22	18	9	11	28	0·996	0·914	1·161	0·982	0·889	0·915
14	26	18	9	11	28	0·897	0·902	1·148	0·947	0·914	0·913
18	22	18	9	12	28	1·000	1·000	1·000	1·000	1·000	1·000
22	22	18	7	7	22	1·192	1·240	0·803	0·888	0·844	0·877
22	26	18	4	2	11	2·260	—	0·891	0·919	—	0·891
Exp. 2											
3	9	22	18	13	8	1·085	0·874	0·849	0·912	0·972	0·800
3	21	22	19	12	7	1·136	0·979	0·826	0·893	0·976	0·884
3	30	22	19	15	8	0·957	0·995	0·587	0·838	0·877	0·680
10	9	22	18	8	19	1·005	0·939	1·029	0·921	0·933	0·951
10	21	22	19	8	18	1·076	0·883	0·880	0·894	0·944	0·952
10	30	22	18	9	19	1·197	0·973	1·054	0·906	0·921	0·893
14	9	22	19	13	19	1·137	1·025	1·040	0·972	0·976	0·961
14	21	22	18	9	19	1·067	0·940	0·863	0·931	0·540	0·923
14	30	22	19	11	17	1·030	0·936	0·763	0·950	0·859	0·907
18	9	22	19	18	20	1·137	0·927	0·903	0·975	0·982	0·937
18	21	22	17	15	19	0·943	0·985	0·861	0·929	0·920	0·940
18	30	22	10	6	11	1·071	0·701	0·761	0·957	0·736	0·924
22	9, 21, 30	22	19	18	23	1·000	1·000	1·000	1·000	1·000	1·000
Precipitation with 50% alcohol											
Exp. 2											
3	9	22	19	12	7	1·001	0·986	0·707	0·884	0·949	0·884
3	21	22	19	14	8	1·059	1·078	0·868	0·841	0·904	0·916
3	30	22	20	15	8	0·859	1·096	0·588	0·832	0·871	0·716
10	9	22	19	8	20	0·902	0·944	0·957	0·901	0·971	0·961
10	21	22	20	9	16	0·953	1·007	0·961	0·861	0·975	0·930
10	30	22	19	9	20	1·025	1·019	1·004	0·874	0·935	0·825
14	9	22	19	12	19	1·075	0·942	0·989	0·949	0·965	0·963
14	21	22	20	9	20	0·965	1·058	0·854	0·938	0·576	0·922
14	30	22	19	9	18	0·981	1·035	0·821	0·958	0·873	0·930
18	9	22	20	17	20	1·031	1·001	0·997	0·972	0·991	0·928
18	21	22	19	16	18	0·866	0·821	0·913	0·927	0·948	0·906
18	30	22	14	6	14	1·086	0·522	0·810	0·953	0·806	0·894
22	9, 21, 30	22	20	18	23	1·000	1·000	1·000	1·000	1·000	1·000
Clot-on-boiling (C.O.B.)											
Exp. 2											
3	9	22	18	11	7	1·013	0·882	0·675	0·879	0·883	0·969
3	21	22	19	14	8	1·078	0·979	0·824	0·881	0·897	0·780
3	30	22	19	15	8	0·902	1·021	0·614	0·841	0·896	0·685
10	9	22	18	8	20	0·965	0·974	0·986	0·919	0·969	0·906
10	21	22	19	8	18	1·005	1·075	0·941	0·874	0·965	0·919
10	30	22	18	9	20	0·997	0·979	1·085	0·903	0·951	0·783
14	9	22	18	13	20	1·047	1·011	0·942	0·944	0·960	0·967
14	21	22	19	9	20	1·014	1·038	0·936	0·948	0·702	0·929
14	30	22	19	10	19	1·017	0·958	0·889	0·942	0·919	0·893
18	9	22	19	17	20	1·067	0·994	0·896	0·973	0·975	0·933
18	21	22	18	16	19	0·890	0·817	0·931	0·932	0·930	0·916
18	30	22	13	6	17	1·083	0·482	0·813	0·960	0·860	0·895
22	9, 21, 30	22	19	18	24	1·000	1·000	1·000	1·000	1·000	1·000

Table 1 (*continued*)

Storage Temp. (° C.)	Storage Time (hr.)	Incubation temp. (° C.)	No. of samples Centre K	No. of samples Centre L	No. of samples Centre M	Value of regression coefficient (*B*) Centre K	Value of regression coefficient (*B*) Centre L	Value of regression coefficient (*B*) Centre M	Value of correlation coefficient (*r*) Centre K	Value of correlation coefficient (*r*) Centre L	Value of correlation coefficient (*r*) Centre M
Exp. 3											
4	7	18	18	20	16	1·010	1·054	0·982	0·980	0·971	0·962
4	7	22	21	21	18	1·084	1·071	0·962	0·965	0·954	0·906
4	7	24	22	21	17	1·205	0·990	0·885	0·970	0·974	0·892
4	17	18	18	20	16	1·303	0·965	0·950	0·937	0·973	0·916
4	17	22	21	21	18	1·198	1·043	1·186	0·962	0·924	0·884
4	17	24	22	21	17	1·430	1·116	1·047	0·973	0·930	0·815
4	23	18	12	17	16	1·737	1·236	1·042	0·897	0·973	0·899
4	23	22	19	21	18	1·303	1·166	1·060	0·962	0·905	0·854
4	23	24	20	21	17	1·337	1·161	0·951	0·931	0·909	0·833
10	7	18	14	16	15	1·152	1·131	0·981	0·977	0·757	0·965
10	7	22	15	16	17	1·221	1·081	1·098	0·985	0·962	0·921
10	7	24	16	16	17	1·261	1·018	0·995	0·985	0·955	0·968
10	17	18	15	16	14	1·243	0·961	1·079	0·969	0·951	0·919
10	17	22	17	15	16	1·152	1·291	1·096	0·982	0·901	0·874
10	17	24	18	16	16	1·326	1·148	1·060	0·942	0·922	0·862
10	23	18	16	15	14	1·325	0·887	1·000	0·945	0·966	0·949
10	23	22	17	16	15	1·235	1·171	1·068	0·974	0·897	0·905
10	23	24	18	17	15	1·406	1·089	0·971	0·943	0·855	0·900
14	7	18	14	18	19	1·013	1·015	0·979	0·968	0·993	0·973
14	7	22	15	19	21	1·049	1·095	1·060	0·993	0·982	0·948
14	7	24	16	19	20	1·199	1·013	1·053	0·989	0·987	0·960
14	17	18	14	18	18	1·075	1·105	0·963	0·990	0·957	0·971
14	17	22	17	19	20	1·231	1·021	1·147	0·961	0·965	0·921
14	17	24	18	19	19	1·290	1·074	1·090	0·966	0·942	0·947
14	23	18	14	19	18	1·157	0·918	0·997	0·979	0·971	0·944
14	23	22	16	19	20	1·199	1·276	1·157	0·986	0·961	0·899
14	23	24	18	20	19	1·292	1·100	1·137	0·971	0·919	0·936
18	7, 17, 23	18	20	20	19	1·000	1·000	1·000	1·000	1·000	1·000
18	7	22	21	20	19	1·054	1·039	1·065	0·991	0·995	0·955
18	7	24	22	20	18	1·039	0·973	0·986	0·959	0·976	0·986
18	17	22	21	21	19	1·047	1·109	0·973	0·978	0·979	0·950
18	17	24	22	21	18	1·176	1·113	1·000	0·967	0·959	0·957
18	23	22	19	21	17	1·131	1·087	1·233	0·974	0·951	0·956
18	23	24	20	21	16	1·266	1·050	0·992	0·931	0·914	0·939
22	7	18	20	19	19	1·042	1·007	1·000	0·961	0·982	0·990
22	7, 17, 23	22	23	21	21	1·000	1·000	1·000	1·000	1·000	1·000
22	7	24	24	21	20	1·006	1·145	0·989	0·965	0·961	0·978
22	17	18	17	20	15	0·970	1·063	1·006	0·981	0·948	0·818
22	17	24	21	21	16	1·050	1·048	0·851	0·978	0·962	0·972
22	23	18	14	15	14	0·832	1·037	1·078	0·952	0·894	0·876
22	23	24	17	17	14	1·055	1·022	1·038	0·936	0·908	0·881
24	7	18	20	19	18	0·856	0·943	0·940	0·985	0·979	0·958
24	7	22	23	21	20	0·982	0·959	0·898	0·989	0·989	0·971
24	7, 17, 23	24	24	21	20	1·000	1·000	1·000	1·000	1·000	1·000
24	17	18	14	18	15	0·851	0·928	1·099	0·941	0·907	0·829
24	17	22	18	19	17	0·904	1·057	0·814	0·968	0·967	0·908
24	23	18	11	11	11	0·874	0·826	1·277	0·951	0·791	0·791
24	23	22	11	12	12	0·842	1·290	0·738	0·955	0·930	0·637
						Methylene blue					
Exp. 3											
4	7	18	18	20	16	1·188	1·041	0·913	0·904	0·973	0·928
4	7	22	21	21	18	1·082	1·058	0·913	0·949	0·957	0·895
4	7	24	22	21	17	0·961	0·969	0·883	0·897	0·947	0·941
4	17	18	18	20	16	1·127	0·967	0·880	0·875	0·971	0·915
4	17	22	21	21	18	1·002	0·990	0·991	0·958	0·966	0·872
4	17	24	22	21	17	1·194	0·907	0·804	0·896	0·925	0·870
4	23	18	16	20	16	1·313	0·989	0·916	0·908	0·937	0·933
4	23	22	19	21	18	1·048	1·151	0·811	0·951	0·884	0·941
4	23	24	20	21	17	1·109	0·973	0·952	0·943	0·942	0·950
10	7	18	14	16	15	1·160	1·053	0·809	0·915	0·964	0·967
10	7	22	15	16	17	1·113	0·951	0·843	0·945	0·952	0·948
10	7	24	16	16	17	1·056	0·978	0·912	0·923	0·930	0·969
10	17	18	16	15	14	1·084	1·138	0·897	0·926	0·957	0·961
10	17	22	17	16	16	1·046	0·927	1·004	0·888	0·917	0·925
10	17	24	18	16	16	1·107	0·985	0·902	0·861	0·969	0·933

Table 1 (*continued*)

Storage Temp. (° C.)	Storage Time (hr.)	Incubation temp. (° C.)	No. of samples Centre K	No. of samples Centre L	No. of samples Centre M	Value of regression coefficient (*B*) Centre K	Value of regression coefficient (*B*) Centre L	Value of regression coefficient (*B*) Centre M	Value of correlation coefficient (*r*) Centre K	Value of correlation coefficient (*r*) Centre L	Value of correlation coefficient (*r*) Centre M
10	23	18	16	17	13	1·139	0·906	0·892	0·911	0·919	0·969
10	23	22	17	17	14	1·220	0·909	0·859	0·848	0·931	0·958
10	23	24	18	17	14	1·141	0·935	0·793	0·860	0·895	0·852
14	7	18	14	18	19	1·123	0·954	0·867	0·968	0·980	0·969
14	7	22	15	19	21	1·061	0·995	0·860	0·926	0·977	0·961
14	7	24	16	19	20	0·874	0·730	0·899	0·906	0·923	0·949
14	17	18	13	18	18	0·986	1·029	0·955	0·963	0·963	0·935
14	17	22	16	19	20	0·936	1·021	0·938	0·873	0·917	0·888
14	17	24	17	19	19	1·165	0·821	0·807	0·729	0·949	0·926
14	23	18	12	17	17	1·176	0·972	0·950	0·940	0·962	0·937
14	23	22	15	18	19	1·023	1·084	0·888	0·903	0·918	0·923
14	23	24	17	18	17	1·116	0·889	0·970	0·755	0·902	0·908
18	7, 17, 23	18	20	20	19	1·000	1·000	1·000	1·000	1·000	1·000
18	7	22	21	20	19	0·958	1·008	0·944	0·974	0·959	0·970
18	7	24	22	20	18	0·928	0·813	0·941	0·949	0·941	0·906
18	17	22	16	18	14	1·059	1·052	0·975	0·930	0·949	0·764
18	17	24	17	18	13	1·036	0·832	0·858	0·894	0·892	0·854
18	23	22	15	15	11	1·210	1·227	0·906	0·835	0·885	0·892
18	23	24	16	16	10	1·377	0·833	1·400	0·773	0·921	0·545
22	7	18	19	20	19	1·168	1·052	1·087	0·930	0·959	0·972
22	7, 17, 23	22	23	21	21	1·000	1·000	1·000	1·000	1·000	1·000
22	7	24	23	21	19	0·948	0·981	0·957	0·890	0·942	0·961
22	17	18	11	13	12	1·042	1·168	1·047	0·752	0·918	0·771
22	17	24	14	13	12	0·960	0·970	1·044	0·848	0·873	0·914
22	23	18	6	6	5	1·839	0·838	1·403	0·699	0·900	0·757
22	23	24	6	5	2	1·608	—	1·500	0·073	—	1·000
24	7	18	19	20	17	0·850	1·098	0·937	0·959	0·972	0·958
24	7	22	21	21	19	0·925	0·955	0·965	0·937	0·982	0·941
24	7, 17, 23	24	24	21	20	1·000	1·000	1·000	1·000	1·000	1·000
24	17	18	6	10	9	0·998	0·516	1·149	0·802	0·800	0·730
24	17	22	6	10	9	1·382	1·292	0·850	0·888	0·745	0·686
24	23	18	3	3	2	3·786	0·577	3·500	0·924	0·866	1·000
24	23	22	3	2	1	1·732	—	—	0·866	—	—
						Methylene blue					
Exp. 3 (tubes not inverted)											
4	7	18	18	16	16	1·208	1·020	0·754	0·974	0·932	0·747
4	7	22	21	21	18	0·958	1·050	0·831	0·862	0·943	0·888
4	7	24	22	21	17	1·095	0·943	0·965	0·926	0·819	0·832
10	7	18	14	14	15	1·151	1·016	0·760	0·972	0·959	0·834
10	7	22	15	16	17	0·898	0·955	0·821	0·890	0·961	0·917
10	7	24	16	15	17	1·065	1·046	0·919	0·931	0·938	0·884
14	7	18	14	16	19	1·414	0·857	1·003	0·850	0·932	0·823
14	7	22	15	19	21	0·712	1·001	0·805	0·896	0·968	0·910
14	7	24	16	18	20	1·296	1·057	0·934	0·927	0·919	0·889
18	7	18	20	18	19	1·000	1·000	1·000	1·000	1·000	1·000
18	7	22	21	20	19	0·741	0·971	0·872	0·936	0·954	0·929
18	7	24	22	19	18	0·833	1·044	0·920	0·937	0·968	0·950
22	7	18	19	18	19	1·056	1·318	1·043	0·924	0·963	0·857
22	7	22	23	21	21	1·000	1·000	1·000	1·000	1·000	1·000
22	7	24	23	20	19	1·008	1·057	0·891	0·956	0·987	0·957
24	7	18	19	18	17	0·912	1·369	1·034	0·972	0·976	0·964
24	7	22	22	21	19	0·840	1·302	1·131	0·922	0·931	0·967
24	7	24	24	21	20	1·000	1·000	1·000	1·000	1·000	1·000
Exp. 4											
4	8	37·5	12	9	12	0·908	1·087	1·028	0·975	0·882	0·967
10	8	37·5	12	7	12	0·926	0·861	0·929	0·965	0·848	0·966
14	8	37·5	12	6	12	0·793	0·930	0·879	0·966	0·879	0·917
18	8	37·5	12	4	12	0·728	0·573	0·866	0·941	0·630	0·836
22	8	37·5	12	4	12	0·915	0·480	0·999	0·940	0·984	0·806
37·5	0	37·5	12	9	12	1·000	1·000	1·000	1·000	1·000	1·000
Exp. 4 (tubes iced overnight)											
4	8	37·5	12	6	12	1·015	0·979	0·860	0·957	0·905	0·978
10	8	37·5	12	6	12	0·898	0·825	0·808	0·946	0·884	0·976
14	8	37·5	12	5	11	0·803	0·773	0·792	0·933	0·857	0·890
18	8	37·5	12	5	11	0·837	0·926	0·808	0·921	0·865	0·815
22	8	37·5	12	4	10	0·939	0·582	0·767	0·943	0·990	0·830
37·5	0	37·5	12	9	12	1·000	1·000	1·000	1·000	1·000	1·000

Further statistical analysis, by regression and correlation, was carried out to obtain more precise information about the temperature compensation curves.

In the experiments one of the subsamples was held during the pre-test storage period at the temperature used subsequently for incubation. The first stage in the analysis was to evaluate the regressions of times to end-point for the series of subsamples stored at the incubation temperature on the times to end-point for each series of subsamples stored at the other temperatures, i.e. for each of the samples in Exp. 1, the regression of A.P.T. for the subsamples stored at 18° C. on that for each of the other sub-samples stored at 4, 10, 14 and 22° C.

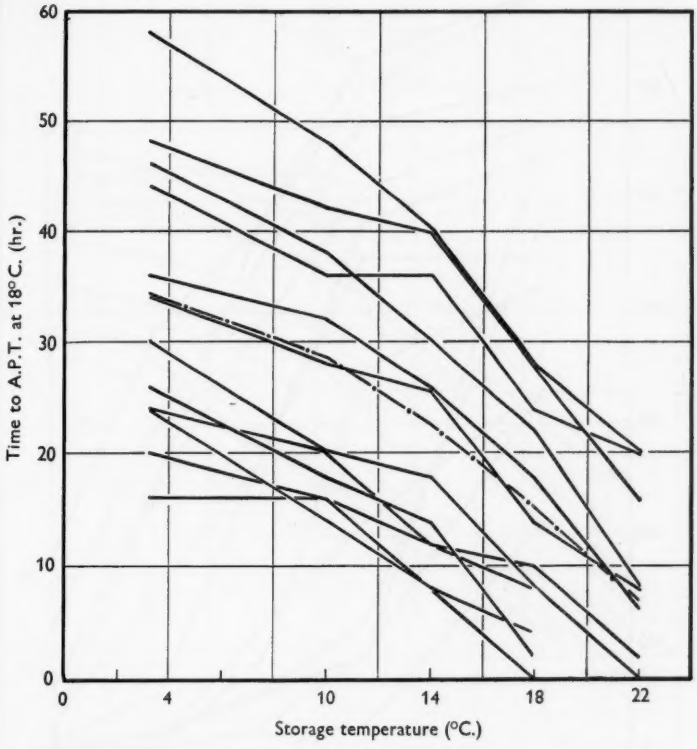

Fig. 1. Time to precipitation with 68% alcohol (A.P.T.) after storage at controlled temperatures for 22 hr. (Exp. 1).

The usual methods of regression analysis were used to evaluate the regression coefficient b_1 in the general equation $y=\bar{y}+b_1(x-\bar{x})$. In the first experiment the variable x is the time to end-point (A.P.T.) at 18° C. after 22 hr. storage at 4, 10, 14, or 22° C. and y is the time to end-point (A.P.T.) at 18° C. after 22 hr. storage at 18° C. Clearly x is subject to error variation in the same way that y is. It would, therefore, be equally valid to evaluate b_2 in the equation $x=\bar{x}+b_2(y-\bar{y})$, but neither b_1 nor b_2 will be the true regression coefficient owing to the presence of the error variation in the variate being used as independent in each case.

In the analysis, therefore, the regression coefficient used will be B, an estimate of the true regression coefficient derived as the geometric mean of b_1 and $1/b_2$, for, to a first

approximation, as shown below, an unbiased estimate of the true regression coefficient can be obtained as the square root of the quotient of the two population regression coefficients.

It is assumed that:

(1) Apart from experimental errors, all milks having a given time θ_1 to end-point after storage at the first temperature have identical times θ_2 to end-point after storage at the second temperature.

(2) The relationship between θ_1 and θ_2 is linear.

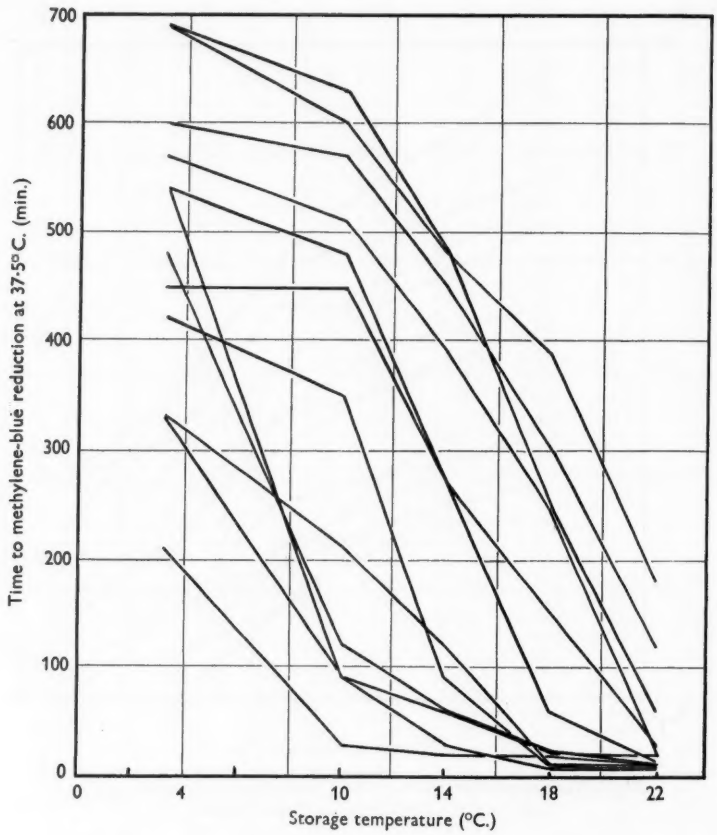

Fig. 2. Time to reduction of methylene blue at 37·5° C. after storage at controlled temperatures for 22 hr. (Exp. 1).

Neither of these conditions will strictly apply, and if the relationship is non-linear the quotient of the two regression coefficients will represent only the ratio of the linear regression coefficients.

Let the true (population) variance of x be $V(x)$ and of (y) be $V(y)$ and the error variance of (x) be $v(x)$ and of y be $v(y)$. Then $V(x)+v(x)$ and $V(y)+v(y)$ are the total population variances of the observations of x and y respectively. Let β_1 be the population regression coefficient of y on x and β_2 the population regression coefficient of x on y, so that b_1 from

the sample estimates β_1 and b_2 estimates β_2. Let β be the true regression coefficient. Then

$$\beta_1=\frac{\text{cov}\,(xy)}{V(x)+v(x)} \quad \text{and} \quad \beta=\frac{\text{cov}\,(xy)}{V(x)},$$

$$\beta_2=\frac{\text{cov}\,(xy)}{V(y)+v(y)} \quad \text{and} \quad \frac{1}{\beta}=\frac{\text{cov}\,(xy)}{V(y)}.$$

Hence

$$\frac{\beta_1}{\beta_2}=\frac{V(y)+v(y)}{V(x)+v(x)} \quad \text{and} \quad \beta^2=\frac{V(y)}{V(x)},$$

giving

$$\beta^2=\frac{\beta_1}{\beta_2}.\frac{1+\dfrac{v(x)}{V(x)}}{1+\dfrac{v(y)}{V(y)}}.$$

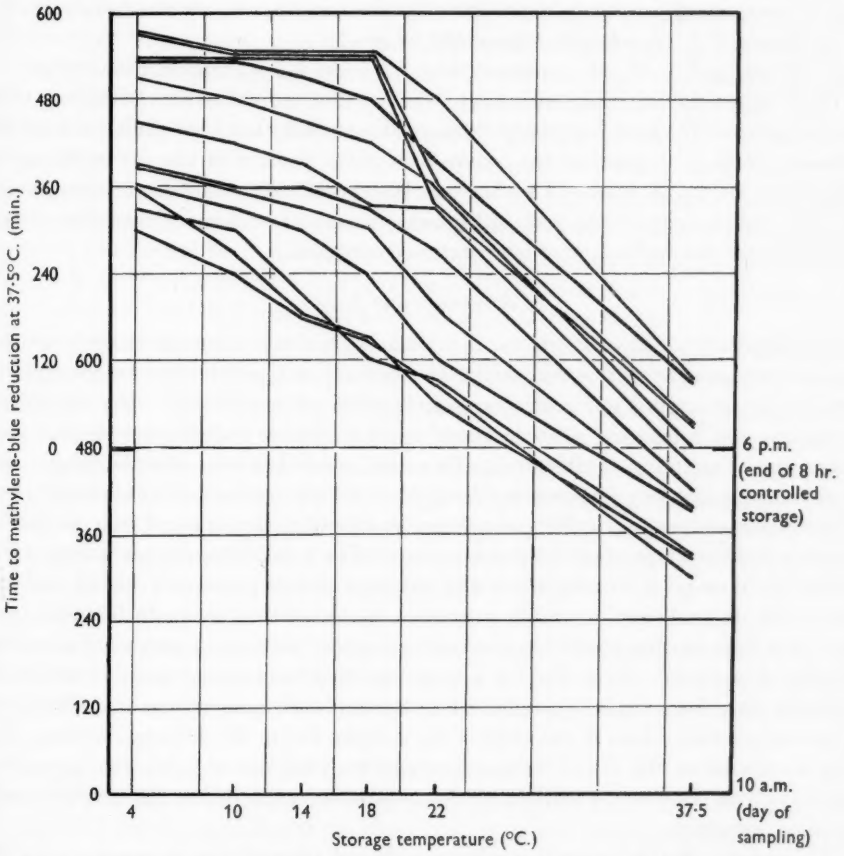

Fig. 3. Time to reduction of methylene blue at 37·5° C. after 0 hr. storage (test begun at 10 a.m.) and after storage at controlled temperatures for 8 hr. (test begun at 6 p.m.) (Exp. 4).

Now we have no knowledge of $V(x)$ and $v(x)$ nor of $V(y)$ and $v(y)$ independently. To estimate β, therefore, some further assumption must be made, and it is considered reason-

able to suppose that the ratio of the error variance to the true variance is equal in both cases, i.e. $\frac{v(x)}{V(x)}=\frac{v(y)}{V(y)}$, so that $\beta^2=\frac{\beta_1}{\beta_2}$.

The estimated regression coefficients B calculated as $\sqrt{\frac{b_1}{b_2}}$, are measures of the spread of the results at one storage temperature compared with the spread of results at the other storage temperature. They therefore indicate whether the temperature compensation curves are, in general, converging, parallel, or diverging. The meaning of these terms may be illustrated as follows. Assume that the observed keeping quality results for n samples are included in the range h_1 hr. after storage at t_1° C., h_2 hr. after storage at t_2° C., h_T hr. after storage at t_T° C., h_3 hr. after storage at t_3° C. where the storage temperature t_T° C. is the same as the incubation temperature and $t_1<t_2<t_T<t_3$.

The curves are then defined as:

(*a*) Converging if $h_1>h_2>h_T$ represented by $B<1$ and $h_3<h_T$ represented by $B>1$.

(*b*) Parallel if $h_1=h_2=h_T=h_3$ represented by $B=1$.

(*c*) Diverging if $h_1<h_2<h_T$ represented by $B>1$ and $h_3>h_T$ represented by $B<1$.

The B values do not, however, indicate whether each individual curve conforms to the general pattern. The most convenient measure of conformity is r, the correlation coefficient between x and y, obtained as the square root of the product of the two regression coefficients b_1 and b_2. A value of 1 for the correlation coefficient will indicate complete conformity; values approaching zero will indicate a lack of conformity rendering the test unsuitable for the application of temperature compensation.

Consideration of figures

The purpose of the experiments was to determine whether it is possible to apply temperature compensation to routine test results. That is to say, is it possible to determine from the result of a test applied to a particular sample, after pre-test storage under the different conditions met in practice, what the result would have been had that sample been tested at the time of sampling, or after storage for a fixed interval at some specified temperature? Such compensation would be accurate if individual samples conformed to a standard pattern of behaviour, and reacted similarly to each combination of storage time and temperature. This requires that the temperature compensation curve of each individual sample be typical of the family of curves given by all samples with the same storage period and that all the curves be parallel. It would not be possible to ensure a continuous and adequate differentiation of one curve from another, should the family of curves tend to cross one another or to converge sharply at one point. From Fig. 1 it may be seen that the keeping quality curves for the different samples are virtually parallel and that there is at all temperatures of pre-test storage a general constancy both in the order of the samples and in the difference between them. Fig. 3 is similar to Fig. 1, and the curves, except for a few minor digressions, appear to be all of one type. However, a markedly different picture is presented in Fig. 2, which requires detailed discussion.

The curves in Fig. 2 suggest that milks are affected differently by the same storage. Thus with some samples an increase in the storage temperature from 4 to 10° C. has no appreciable effect on the reduction time, whereas with others the effect is marked. With a storage temperature of 18° C., or higher, no adequate differentiation between samples is possible except for the best milks. Of the samples in Fig. 2, six reduced methylene blue within

20 min. of the addition of the dye after storage at 18° C.; for the same samples stored at 10° C., the reduction times are 30, 90, 90, 120, 210 and 360 min.; and after storage at 3·6 ° C. they are 210, 330, 540, 480, 330 and 420 min. respectively. It appears from the results that it is not possible to predict a result after storage at a particular temperature from the result obtained after storage at any other temperature. It must, however, be emphasized that these remarks are based on results obtained only after a period of storage of 22 hr.

Further, from Fig. 2, there is a suggestion that there are two types of curves—those for the good milks with a reduction time of 60 min. or more after storage at 22° C. for 22 hr., and those for the bad milks. The curves for the good milks appear to be regular in much the same way as with methylene-blue curves at 37·5° C. after 8 hr. storage as shown in Fig. 3. It may be envisaged, therefore, that good milks could be represented by a single curve, and that in consequence it should be possible to apply temperature compensation to methylene-blue results obtained even after long periods of storage at relatively high temperatures. This constancy of the curves for good milks is perhaps not unexpected, bearing in mind the specialized character of their flora at the time of reduction of methylene blue at 37·5° C. (Wilson (12), Hobbs (15), Rowlands & Garvie (16)). The bad milks are represented mainly by curves which are concave or sigmoidal in shape. It may be that the shape of these curves, particularly in the region represented by storage at 18 and 22° C., is to some extent affected by the nature of the methylene-blue test itself. It is conceivable that samples with a reduction time of 30 min., or less, would have taken the same time to decolorize had the dye been added after a storage period of less than 22 hr., that is to say it may not be possible to differentiate between milks which had only just reached this point and those which had reached it some considerable time earlier.

That the curves should follow a systematic course, preferably parallel, for a fixed period of storage is necessary for accurate temperature compensation. That they should represent the same rate of deterioration for different storage periods is necessary for the general application of temperature compensation to milks of varying ages. These points have already been stressed. Clearly, from Fig. 2, it is not possible to devise a satisfactory temperature compensation formula which would be applicable to all samples after storage for 22 hr. at temperatures within the range studied (4–22° C.); but it may be that with a shorter period of storage all milks would present curves of the same degree of regularity (Fig. 3) as those given only by the good milks after storage for 22 hr. Thus the temperature compensation of this test would have only limited application. In this connexion it is of interest that Morton & Vincent (9) reached a similar conclusion and noted that adequate differentiation between samples with the methylene-blue test at 37·5° C. was only possible provided the storage period was not excessive and the storage temperature not too high.

The regression coefficient B

In all the experiments for every combination of storage time and temperature there is one value of B for the regression of the times to end-point of subsamples stored at the incubation temperature on those stored at every other temperature. Thus for C.O.B. in Exp. 2 there are in all thirty-six values of B, i.e. 22/3, 22/10, 22/14, 22/18° C. for each storage period of 9, 21 and 30 hr., and for each of the centres K, L, M.

All the values of B for the different experiments are given in Table 1, and the summarized results in Table 2.

Keeping quality measured by C.O.B. Clot-on-boiling was used as the end-point of keeping quality in Exps. 2 and 3. Table 2 has been given in two sections because when the pre-test storage temperature is lower than the incubation temperature, values for B in excess of unity indicate diverging* temperature compensation curves, but converging* curves if the pre-test storage temperature is higher than the incubation temperature.

The figures in Table 2 clearly show a difference between the values of B in the two experiments. In Exp. 2 the results tend to be less than unity, whereas in Exp. 3 they tend to be greater than unity. There are also differences between the centres in both experiments, centre K having the highest and centre L the lowest average values (see detailed figures, Table 1). However, analysis of variance did not indicate any consistent effect of the pre-test storage or incubation conditions on the values of B for the three centres.

The mean value of B is 1·06 indicating that the temperature compensation curves for C.O.B. may, on the average, be regarded as parallel. The experimental values of B show that slight divergence from parallelism may be expected for tests carried out in different areas, and at different times in the same area.

Keeping quality measured by A.P.T. A.P.T. with 68% alcohol was used as the end-point of keeping quality in Exps. 1 and 2, and with 50% alcohol in Exp. 2.

The average value of B for the three centres in Exp. 1 was 0·95; the average was 0·93 for the winter samples (5 November 1945–18 March 1946), and 0·97 for the early summer samples (26 March–24 June 1946). In Exp. 2 the average value was 0·96 for 68% alcohol, and 0·94 for 50% alcohol.

Once again there is no important departure from unity and the analysis of variance indicated no consistent effect of either temperature or duration of pre-test storage at each centre.

Methylene-blue test with incubation at 18, 22 *and* 24° C. The methylene-blue test with incubation at 18, 22 and 24° C. was carried out on subsamples in Exp. 3. The results are exactly comparable with those of the C.O.B. test in this experiment as both tests were applied to subsamples of the same milks after identical pre-test storage.

As with the values of B for the C.O.B. test there are differences between centres, centre K giving the highest values and centre L the lowest (Table 1). The values for the methylene-blue test are slightly lower than those for the comparable C.O.B. results, the average for the former being 0·97 and for the latter 1·09.

The average values of B for subsamples tested after 7 hr. pre-test storage with or without inversion of the methylene-blue tubes at each observation are 0·97 and 0·95 respectively. It may therefore be concluded that inversion of the tubes, in the methylene-blue test at temperatures of 18, 22 and 24° C., has no important effect on the values of B.

Methylene-blue test at 37·5° C. The methylene-blue test was carried out on samples in Exp. 1 after storage for 22 hr. at 4, 10, 14, 18 and 22° C. No subsample was stored at the temperature of incubation, 37·5° C., and consequently an analysis, strictly comparable to that used for other results, could not be carried out. In view of the conclusions drawn from the inspection of the temperature compensation curves for this data (p. 57), no useful purpose would be served in offering an alternative analysis as, although various estimates of B and r could be obtained, they could not be considered reliable and might even be misleading.

A limited amount of information on the effect of storage for a short period on the

* For an explanation of the terms converging and diverging, see p. 56.

Table 2. *Classified distribution of the values of the regression coefficient (B), showing the number of results in each class: results combined for centres K, L, M*

Test	Exp.	Range of values of B: 0·774 and less	0·775 to 0·824	0·825 to 0·874	0·875 to 0·924	0·925 to 0·974	0·975 to 1·024	1·025 to 1·074	1·075 to 1·124	1·125 to 1·174	1·175 to 1·224	1·225 to 1·274	1·275 and over	Total
Values of (B) when storage temperature is less than incubation temperature														
Clot-on-boiling (C.O.B.)	2	3	3	0	5	7	11	3	4	0	0	0	0	36
	3	0	0	1	3	9	19	22	15	13	7	7	12	108
Precipitation with 68% alcohol (A.P.T.)	1	0	0	4	6	5	2	0	3	3	1	0	0	24
	2	4	0	5	3	7	4	7	2	3	1	0	0	36
Precipitation with 50% alcohol	2	3	3	4	2	6	9	5	4	0	0	0	0	36
Methylene blue at 18, 22, 24° C.	3 (tubes inverted)	1	7	8	16	23	14	12	8	7	5	1	4	106*
	3 (tubes not inverted)	4	2	4	4	6	5	6	1	1	1	0	2	36
Methylene blue at 37·5° C.	4	3	1	2	3	3	1	1	1	0	0	0	0	15
	4 (iced overnight)	3	4	3	1	2	2	0	0	0	0	0	0	15
Values of (B) when storage temperature is greater than incubation temperature														
Clot-on-boiling (C.O.B.)	3	1	1	6	2	5	4	4	2	0	0	0	2	27
Precipitation with 68% alcohol (A.P.T.)	1	1	1	0	2	1	0	1	0	0	2	1	2	11*
Methylene blue at 18, 22, 24° C.	3 (tubes inverted)	2	0	3	0	4	1	3	2	3	0	0	6	24*
	3 (tubes not inverted)	0	0	1	1	0	0	3	0	1	0	0	3	9

* Excluding (B) values calculated from less than three subsamples.

methylene-blue test at 37·5° C. was obtained from Exp. 4. In this experiment a single subsample was tested by methylene blue at 37·5° C. at the time storage was begun and served as a key sample for the calculation of B and r values: in addition, five pairs of subsamples were placed under controlled storage, one of each pair being tested after 8 hr. and the other after a further period of 16 hr. in the refrigerator. The effect of refrigeration varied slightly but, in general, served only to change the reduction time by about half an hour.

The mean values of the regression coefficients B for the relationships 4/37·5, 10/37·5, 14/37·5, 18/37·5, 22/37·5° C. are 1·01, 0·91, 0·87, 0·72 and 0·80 respectively. These suggest that the curves are not parallel and this is supported by the fact that the mean B value is 0·86, and is different from 1 with a high level of significance. This being so the curves must be regarded as converging. This means, first, that the difference between milks become less well determined as the storage temperature increases. This effect is similar to that obtained in Exp. 1, with storage for 22 hr., but is less pronounced and unlike the results then obtained; the curves for different samples do not become confused even at the highest storage temperatures. Secondly, temperature compensation must vary according to the level of bacteriological quality of the milk so that the mean temperature compensation curve is of little more than academic interest.

The correlation coefficient r

For each regression coefficient B, one value of the correlation coefficient r is obtained. Table 3 shows the classified distribution of the values of r; complete results are given in Table 1.

The results for the C.O.B. test show a high degree of correlation, i.e. r is close to unity; the r values are only slightly lower for the alcohol test, and for the methylene-blue test with incubation at 18, 22 and 24° C. With the methylene-blue test at 37·5° C. (Exp. 4) the values are as high as for any of the other tests investigated. These high values indicate that there is no serious crossing over of the various temperature compensation curves.

It is concluded that with the C.O.B. test, the alcohol test using either 68% or 50% alcohol, and the methylene-blue test with incubation at 18, 22 or 24° C., the regression coefficient B can be regarded as unity, and the ranges of the times to end-point after pre-test storage at temperatures under review (4–24° C.) as equal. Furthermore, especially with the C.O.B. test, there is little intersection among the individual temperature compensation curves. These curves may, therefore, be treated as parallel. Consequently, it should be possible to design temperature compensation scales for these tests, which would adjust to a common basis the results for samples tested after preliminary storage for different times at different temperatures.

These conclusions do not apply unconditionally to the results obtained with the methylene-blue test at 37·5° C. carried out after storage for a period of 8 hr. Because of the convergence of the temperature compensation curves, a similar compensation scheme does not apply, although this does not preclude the possibility of devising one if available results were adequate.

Table 3. *Classified distribution of the values of the correlation coefficient (r) showing the number of results in each class: results combined for centres K, L, M*

Test	Exp.	Range of values of (r): 1·000 to 0·950	0·949 to 0·900	0·899 to 0·850	0·849 to 0·800	0·799 to 0·750	0·749 to 0·700	0·699 to 0·650	0·649 to 0·600	0·599 to 0·550	0·549 to 0·500	0·499 and less	Total
Clot-on-boiling (C.O.B.)	2	9	13	9	1	2	1	1	0	0	0	0	36
	3	79	35	13	4	3	0	0	1	0	0	0	135
Precipitation with 68% alcohol (A.P.T.)	1	10	13	8	2	1	0	0	0	0	0	1	35*
	2	10	15	6	2	0	1	1	0	0	1	0	36
Precipitation with 50% alcohol	2	9	14	7	4	0	1	0	0	1	0	0	36
Methylene blue at 18, 22, 24° C.	3 (tubes inverted)	33	55	24	5	6	3	2	0	0	1	1	130*
	3 (tubes not inverted)	16	16	7	5	0	1	0	0	0	0	0	45
Methylene blue at 37·5° C.	4	6	3	2	3	0	0	0	1	0	0	0	15
	4 (iced overnight)	4	5	4	2	0	0	0	0	0	0	0	15

* Excluding (r) values calculated from less than three subsamples.

Table 4. *Ratio of rate of deterioration at storage temperature to rate of deterioration at incubation temperature, r(t)*

Clot-on-boiling (C.O.B.)

Incubation temperature (° C.)

Exp.	Storage period (hr.)	Centre	18 Pre-test storage temperature (° C.) 3–4	10	14	18	22	24	22 Pre-test storage temperature (° C.) 3–4	10	14	18	22	24	24 Pre-test storage temperature (° C.) 3–4	10	14	18	22	24
3	7	*K*	0·17	0·45	0·77	1·00	1·54	1·54	0·09	0·29	0·68	0·73	1·00	1·11	0·21	0·28	0·63	0·66	0·80	1·00
		L	0·05	0·43	0·62	1·00	1·42	1·67	0·01	0·14	0·39	0·66	1·00	1·14	0·03	0·12	0·39	0·61	0·95	1·00
		M	0·18	0·36	0·59	1·00	1·47	1·71	0·09	0·17	0·41	0·64	1·00	1·18	0·05	0·16	0·36	0·57	0·89	1·00
		Mean	0·13	0·41	0·66	1·00	1·47	1·64	0·06	0·20	0·49	0·68	1·00	1·14	0·10	0·18	0·46	0·61	0·88	1·00
2	9	*K*	—	—	—	—	—	—	0·06	0·22	0·43	0·73	1·00	—	—	—	—	—	—	—
		L	—	—	—	—	—	—	0·14	0·39	0·50	0·75	1·00	—	—	—	—	—	—	—
		M	—	—	—	—	—	—	−0·05	0·14	0·32	9·63	1·00	—	—	—	—	—	—	—
		Mean	—	—	—	—	—	—	0·05	0·25	0·41	0·71	1·00	—	—	—	—	—	—	—
3	17	*K*	0·15	0·35	0·62	1·00	1·41	1·61	0·13	0·23	0·53	0·69	1·00	1·10	0·17	0·27	0·48	0·64	0·87	1·00
		L	−0·01	0·21	0·62	1·00	1·44	1·71	0·01	0·21	0·36	0·67	1·00	1·20	0·03	0·18	0·36	0·57	0·86	1·00
		M	0·06	0·37	0·57	1·00	1·47	1·73	0·06	0·19	0·39	0·65	1·00	1·03	0·01	0·17	0·34	0·59	0·81	1·00
		Mean	0·07	0·31	0·61	1·00	1·44	1·68	0·06	0·21	0·43	0·67	1·00	1·11	0·07	0·20	0·39	0·60	0·85	1·00
2	21	*K*	—	—	—	—	—	—	0·04	0·22	0·47	0·69	1·00	—	—	—	—	—	—	—
		L	—	—	—	—	—	—	0·09	0·27	0·44	0·71	1·00	—	—	—	—	—	—	—
		M	—	—	—	—	—	—	0·02	0·19	0·39	0·68	1·00	—	—	—	—	—	—	—
		Mean	—	—	—	—	—	—	0·05	0·23	0·43	0·69	1·00	—	—	—	—	—	—	—
3	23	*K*	0·24	0·38	0·68	1·00	1·33	1·55	0·16	0·25	0·47	0·73	1·00	1·07	0·17	0·25	0·49	0·63	0·89	1·00
		L	0·07	0·27	0·54	1·00	1·42	1·64	0·06	0·17	0·43	0·69	1·00	1·21	0·04	0·20	0·36	0·58	0·84	1·00
		M	0·09	0·34	0·57	1·00	1·42	1·57	0·07	0·24	0·42	0·67	1·00	1·03	0·07	0·23	0·37	0·57	0·84	1·00
		Mean	0·13	0·33	0·60	1·00	1·39	1·58	0·10	0·22	0·44	0·70	1·00	1·11	0·09	0·23	0·41	0·59	0·86	1·00
2	30	*K*	—	—	—	—	—	—	0·01	0·22	0·44	0·70	1·00	—	—	—	—	—	—	—
		L	—	—	—	—	—	—	0·10	0·28	0·42	0·68	1·00	—	—	—	—	—	—	—
		M	—	—	—	—	—	—	0·01	0·21	0·42	0·68	1·00	—	—	—	—	—	—	—
		Mean	—	—	—	—	—	—	0·04	0·24	0·43	0·69	1·00	—	—	—	—	—	—	—

Precipitation with 68% alcohol (A.P.T.)

Incubation temperature (° C.)

Exp.	Storage period (hr.)	Centre	18 Pre-test storage temperature (° C.) 3–4	10	14	18	22	24	22 Pre-test storage temperature (° C.) 3–4	10	14	18	22	24	**Precipitation with 50% alcohol Incubation temperature, 22° C.** Pre-test storage temperature (° C.) 3–4	10	14	18	22	24
2	9	*K*	—	—	—	—	—	—	0·14	0·27	0·56	0·75	1·00	—	0·05	0·21	0·44	0·72	1·00	—
		L	—	—	—	—	—	—	0·11	0·33	0·47	0·73	1·00	—	0·15	0·38	0·49	0·75	1·00	—
		M	—	—	—	—	—	—	0·00	0·15	0·37	0·73	1·00	—	−0·02	0·17	0·36	0·69	1·00	—
		Mean	—	—	—	—	—	—	0·08	0·25	0·47	0·74	1·00	—	0·06	0·25	0·43	0·72	1·00	—
2	21	*K*	—	—	—	—	—	—	0·08	0·23	0·47	0·71	1·00	—	0·05	0·20	0·46	0·68	1·00	—
		L	—	—	—	—	—	—	0·12	0·28	0·45	0·70	1·00	—	0·12	0·30	0·46	0·72	1·00	—
		M	—	—	—	—	—	—	0·07	0·25	0·42	0·73	1·00	—	0·02	0·17	0·38	0·69	1·00	—
		Mean	—	—	—	—	—	—	0·09	0·25	0·45	0·72	1·00	—	0·07	0·23	0·43	0·70	1·00	—
1 (winter)	22	*K*	0·03	0·36	0·63	1·00	1·40	—	—	—	—	—	—	—	—	—	—	—	—	—
		L	0·08	0·37	0·58	1·00	1·35	—	—	—	—	—	—	—	—	—	—	—	—	—
		M	0·23	0·42	0·67	1·00	1·33	—	—	—	—	—	—	—	—	—	—	—	—	—
		Mean	0·11	0·38	0·63	1·00	1·36	—	—	—	—	—	—	—	—	—	—	—	—	—
1 (summer)	22	*K*	0·18	0·38	0·71	1·00	1·43	—	—	—	—	—	—	—	—	—	—	—	—	—
		L	0·14	0·39	0·67	1·00	1·38	—	—	—	—	—	—	—	—	—	—	—	—	—
		M	0·22	0·44	0·73	1·00	1·33	—	—	—	—	—	—	—	—	—	—	—	—	—
		Mean	0·18	0·40	0·70	1·00	1·38	—	—	—	—	—	—	—	—	—	—	—	—	—
1 (winter)	26	*K*	—	—	0·63	1·00	1·37	—	—	—	—	—	—	—	—	—	—	—	—	—

1 (summer)	22	*K*	0·18	0·38	0·71	1·00	1·43	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		L	0·14	0·39	0·67	1·00	1·38	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		M	0·22	0·44	0·73	1·00	1·33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		Mean	0·18	0·40	0·70	1·00	1·38	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1 (winter)	26	*K*	—	—	0·63	1·00	1·37	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		L	—	—	0·54	1·00	1·39	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		M	—	—	0·65	1·00	1·35	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		Mean	—	—	0·61	1·00	1·37	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1 (summer)	26	*K*	—	—	0·68	1·00	1·37†	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		L	—	—	0·61	1·00	1·46†	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		M	—	—	0·71	1·00	1·29	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		Mean	—	—	0·67	1·00	1·37	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2	30	*K*	—	—	—	—	—	—	0·03	0·21	0·47	0·71	1·00	—	0·01	0·22	0·44	0·71	1·00	—			
		L	—	—	—	—	—	—	0·12	0·26	0·45	0·66	1·00	—	0·11	0·26	0·45	0·67	1·00	—			
		M	—	—	—	—	—	—	0·05	0·24	0·44	0·67	1·00	—	0·02	0·21	0·43	0·69	1·00	—			
		Mean	—	—	—	—	—	—	0·07	0·24	0·45	0·68	1·00	—	0·05	0·23	0·44	0·69	1·00	—			

Methylene blue

			Incubation temperature (° C.)																	
			18 Pre-test storage temperature (° C.)						22 Pre-test storage temperature (° C.)						24 Pre-test storage temperature (° C.)					
Exp.	Storage period (hr.)	Centre	3–4	10	14	18	22	24	3–4	10	14	18	22	24	3–4	10	14	18	22	24
3	7	*K*	−0·33	0·08	0·42	1·00	1·45	1·59	−0·12	−0·06	0·43	0·67	1·00	1·18	−0·40	−0·13	0·20	0·44	0·89	1·00
		L	0·01	0·20	0·62	1·00	1·58	1·81	−0·10	0·01	0·35	0·72	1·00	1·12	−0·16	0·01	0·12	0·40	0·84	1·00
		M	−0·13	0·06	0·50	1·00	1·41	1·53	−0·14	0·09	0·39	0·66	1·00	1·11	−0·01	0·19	0·40	0·55	0·89	1·00
		Mean	−0·15	0·11	0·51	1·00	1·48	1·64	−0·12	0·01	0·39	0·69	1·00	1·14	−0·19	0·03	0·24	0·46	0·87	1·00
3	17	*K*	−0·05	0·23	0·55	1·00	1·39	1·56	−0·09	0·12	0·42	0·65	1·00	1·24	−0·09	0·06	0·25	0·55	0·77	1·00
		L	−0·05	0·21	0·60	1·00	1·52	1·62	−0·08	0·12	0·37	0·66	1·00	1·23	−0·09	0·08	0·29	0·56	0·80	1·00
		M	−0·09	0·17	0·46	1·00	1·41	1·49	−0·05	0·16	0·36	0·70	1·00	0·98	−0·05	0·16	0·28	0·57	0·88	1·00
		Mean	−0·06	0·20	0·54	1·00	1·44	1·56	−0·08	0·13	0·38	0·67	1·00	1·15	−0·08	0·10	0·28	0·56	0·82	1·00
3	23	*K*	0·02	0·23	0·61	1·00	1·30	1·48†	0·00	0·16	0·40	0·66	1·00	1·13†	−0·05	0·09	0·34	0·54	0·80	1·00
		L	−0·04	0·20	0·52	1·00	1·54	1·74†	−0·04	0·09	0·36	0·70	1·00	1·52†	−0·05	0·09	0·27	0·52	0·77	1·00
		M	−0·02	0·27	0·50	1·00	1·23	1·20†	0·00	0·20	0·38	0·73	1·00	1·00†	0·02	0·16	0·39	0·60	0·80†	1·00
		Mean	−0·01	0·23	0·54	1·00	1·36	1·47	−0·01	0·15	0·38	0·69	1·00	1·22	−0·03	0·11	0·33	0·55	0·79	1·00
3 (tubes not inverted)	7	*K*	−0·57	−0·16	0·85	1·00	1·54	1·79	−0·67	−0·37	−0·17	0·27	1·00	1·18	−0·54	−0·31	0·04	0·16	0·85	1·00
		L	−0·88	−0·13	0·12	1·00	2·02	2·36	−0·47	−0·47	0·19	0·61	1·00	1·49	−0·69	−0·34	0·14	0·55	0·91	1·00
		M	−1·17	−0·49	0·50	1·00	1·43	1·88	−0·83	−0·44	−0·04	0·48	1·00	1·23	−0·66	−0·31	0·06	0·52	0·68	1·00
		Mean	−0·87	−0·26	0·49	1·00	1.66	2·01	−0·66	−0·43	−0·01	0·45	1·00	1·30	−0·63	−0·32	0·08	0·41	0·82	1·00

			Incubation temperature (° C.)																	
			37·5 Pre-test storage temperature (° C.)												37·5 Pre-test storage temperature (° C.)					
			3–4	10	14	18	22	37·5							3–4*	10*	14*	18*	22*	37·5
4	8	*K*	−0·07	−0·02	0·04	0·13	0·32	1·00	—	—	—	—	—	—	−0·03	0·02	0·07	0·16	0·29	1·00
		L	0·10	0·22	0·23	0·41†	0·39†	1·00	—	—	—	—	—	—	0·10	0·19	0·26	0·34	0·47†	1·00
		M	0·04	0·12	0·25	0·38	0·51	1·00	—	—	—	—	—	—	0·04	0·13	0·30	0·44	0·57	1·00
		Mean	0·02	0·11	0·17	0·30	0·41	1·00	—	—	—	—	—	—	0·04	0·11	0·21	0·31	0·44	1·00

* Controlled storage at temperature quoted for 8 hr. followed by 16 hr. in refrigerator which has been ignored in the calculation of these $r(t)$ values.

† $r(t)$ values calculated from less than five subsamples.

THE RATE OF DETERIORATION OF MILK DURING PRE-TEST STORAGE

If the individual curves can be regarded as parallel, each will be parallel to the mean curve. The next step in the analysis, therefore, is to estimate the effect of pre-test storage on the mean curve. Suppose that during pre-test storage at any temperature $t°$ C. milk is deteriorating at a rate $r(t)$ times as fast as it would if it were being stored at the incubation temperature $T°$ C. That is to say 1 hr. storage at temperature $t°$ C. prior to incubation at $T°$ C. is equivalent to $r(t)$ hr. storage at $T°$ C. On this basis it is possible to evaluate $r(t)$ for each of the pre-test storage conditions used in the experiments from the general equation $h \times r(t) + \bar{x}_t = h + \bar{x}_T$, where $\bar{x}_t$ is the mean of the times to end-point at $T°$ C. after h hr. storage at temperature $t°$ C., and $\bar{x}_T$ is the mean of the times to end-point at $T°$ C. after h hr. storage at $T°$ C. Hence

$$r(t) = 1 - \frac{\bar{x}_t - \bar{x}_T}{h}.$$

Values of $\bar{x}_t - \bar{x}_T$ have been calculated for all the different conditions of storage. Some of the milk samples reached end-point before the end of storage where the storage temperature was above the incubation temperature. These samples have been omitted when calculating the appropriate values of $\bar{x}_t - \bar{x}_T$.

Consideration of the $r(t)$ values, Table 4, shows that in the first experiment where there was seasonal differentiation, summer values were slightly higher than those for winter. In all the experiments there were differences in overall values between the centres. The analysis of variance, however, shows that the 'Storage Temperature × Centres' and the 'Length of Storage × Centres' interactions are not significant; and the conclusion from this analysis is that differences between centres in the effects of changes in temperature and duration of storage could have arisen by chance.

The values of $\bar{x}_t - \bar{x}_T$ have therefore been averaged over centres to calculate mean values of $r(t)$ which are given in Table 4, together with the detailed results, for each condition of storage and incubation used in the three experiments.

Inspection of the results for the C.O.B. test shows that $r(t)$ varies with the incubation temperature and with the storage temperature, but it is not affected by the duration of storage. Values for incubation at 22° C. are obtained from both the second and the third experiments and they agree very closely.

The values obtained for the A.P.T. in the first and second experiments agree very closely with the C.O.B. values.

The values of $r(t)$ obtained for the methylene-blue test at 18, 22 and 24° C. in the third experiment do not, however, follow quite the same pattern. Where the storage temperature is high they agree well with the results obtained with the other tests. At the lower storage temperatures, however, the values of $r(t)$ are rather lower than with C.O.B. or A.P.T., and with storage at 4° C. they are consistently negative. This means that storage at this temperature actually increases the time to end-point with the methylene-blue test. Further, unlike C.O.B. or A.P.T., this test appears to be affected by the duration of storage at low temperatures and the values of $r(t)$ are distinctly lower with short periods of storage than with long. Other workers (Frayer (13); Wilson (12), Phillips & Thomas (14), Smythe (7)) have observed, on the average, a lengthening of the methylene-blue reduction time at 37·5° C. following storage of milk samples at temperatures of the order of 5° C. or lower.

In the third experiment with the subsamples which were not inverted at the time of observation, the increase in reduction time after storage at low temperatures is even more marked and negative values of $r(t)$ are obtained after storage at 10° C. as well as 4° C. In addition, with these same subsamples the values of $r(t)$ are very high in comparison with other $r(t)$ values obtained when the storage temperature exceeds the incubation temperature.

It must be concluded that the methylene-blue test at 18, 22 or 24° C. measures some factor in milk which is not identical with that measured by C.O.B. or A.P.T., since the effect of pre-test storage conditions is different. This difference is accentuated when the methylene-blue tubes are not inverted.

Because of the convergence of the family of curves representing methylene-blue at 37·5° C., the mean curve cannot be considered typical of the behaviour exhibited by all milks. However, as a matter of interest, values of $r(t)$ were estimated in the same way as for the other tests. Though the mean $r(t)$ values were found to agree well with the theoretical values of $R(t)=\left(\frac{t+3}{T+3}\right)^2$, derived below, those for each centre presented marked individual characteristics. While those for centre *K* were consistently lower than the theoretical values and in some measure supported the comments made above about $r(t)$ values for methylene blue at 18, 22 and 24° C., those for centre *M* were markedly higher than expectation. Centre *L*, too, had very high $r(t)$ values for the lower storage temperatures; at higher temperatures the values were very variable.

A GENERAL FORMULA FOR THE RATE OF DETERIORATION

For each incubation temperature, the series of $r(t)$ values (Table 5—average all periods) were graphed. The result was a set of curves for A.P.T. almost identical with those for C.O.B. at the same incubation temperature. The curves appear tangential to the t axis at about -3° C. and, by definition, pass through unity when the storage temperature t is equal to the incubation temperature T. The simplest curve of this type is the parabola, and the parabola which passes through the two points defined above is given by $R(t)=\left(\frac{t+3}{T+3}\right)^2$. In Table 5 the mean observed values of $r(t)$ for C.O.B. and for precipitation time with 68 and 50% alcohol are compared with the corresponding values of $r(t)$ obtained from the theoretical parabola $R(t)=\left(\frac{t+3}{T+3}\right)^2$. This comparison is shown graphically in Fig. 4.

The agreement is excellent.

The conclusion is, therefore, that if a sample of milk is stored at a temperature t° C. prior to incubation at T° C. until end-point with C.O.B. or A.P.T., each hour of this storage is equivalent to $\left(\frac{t+3}{T+3}\right)^2$ hr. of incubation at T° C. This appears to be applicable for any conditions of storage and incubation within the range covered in the experiments.

It can be seen from the values of $r(t)$ in Table 4 that this formula cannot be applied where the methylene-blue test is used with incubation at 18, 22 or 24° C. Reference has already been made to the difference between $r(t)$ values for keeping quality and dye tests. However, on graphing the series of $r(t)$ values for the methylene-blue test at these temperatures a further set of curves was obtained, again apparently parabolic in shape and, by definition, passing through unity when the storage temperature t° C. is equal to

Table 5. *Comparison of the observed values—taken as the mean over centres and storage periods—of r(t) for clot-on-boiling* (C.O.B.) *and precipitation with* 68% (A.P.T.) *or* 50% *alcohol, with the values obtained from the formula* $R(t)=\left(\frac{t+3}{T+3}\right)^2$

Storage temperature (° C.)	Incubation temperature (T° C.) 18 r(t) C.O.B.	68% alcohol	50% alcohol	R (t)	22 r (t) C.O.B.	68% alcohol	50% alcohol	R (t)	24 r (t) C.O.B.	68% alcohol	50% alcohol	R(t)
3	—	—	—	0·08	0·05	0·08	0·06	0·06	—	—	—	0·05
4	0·11	0·15	—	0·11	0·07	—	—	0·08	0·09	—	—	0·07
10	0·35	0·39	—	0·38	0·22	0·25	0·24	0·27	0·21	—	—	0·23
14	0·62	0·65	—	0·66	0·44	0·45	0·44	0·46	0·42	—	—	0·40
18	1·00	1·00	—	1·00	0·69	0·71	0·70	0·71	0·60	—	—	0·60
22	1·43	1·37	—	1·42	1·00	1·00	1·00	1·00	0·86	—	—	0·86
24	1·63	—	—	1·65	1·12	—	—	1·17	1·00	—	—	1·00

Table 6. *Distribution of differences between observed and calculated times to precipitation with* 68% *alcohol* (A.P.T.) *at* 18 *and* 22° C. *for samples stored for* 22, 9, 21 *and* 30 *hr.; and to clot-on-boiling* (C.O.B.) *at* 22° C. *for samples stored for* 9, 21 *and* 30 *hr.*

Test	Exp.	Storage Period (hr.)	Observed minus calculated times to end-point (hr.). No. of samples in each range: −4·50 and less	−3·50 to −4·49	−2·50 to −3·49	−1·50 to −2·49	−0·50 to −1·49	−0·49 to 0·49	0·50 to 1·49	1·50 to 2·49	2·50 to 3·49	3·50 to 4·49	4·50 to 5·49	5·50 and over	Total
A.P.T. 68% alcohol	1	22	12	4	10	13	19	13	22	11	6	4	8	11	133
A.P.T. 68% alcohol	2	9	1	0	2	5	6	12	19	11	1	0	0	0	57
A.P.T. 68% alcohol	2	21	1	1	1	4	9	8	10	6	9	1	0	0	50
A.P.T. 68% alcohol	2	30	1	0	4	7	3	9	6	5	2	1	0	1	39
C.O.B.	2	9	1	0	2	8	11	11	16	2	1	4	0	0	56
C.O.B.	2	21	1	2	4	6	8	9	11	8	2	2	0	0	53
C.O.B.	2	30	2	1	8	7	3	8	6	5	1	1	2	0	44

the incubation temperature $T°$ C.: but unlike the set of curves for C.O.B. and A.P.T. the vertices of the parabolas were below the t axis—$r(t)$ approximately $-0{\cdot}12$—and appear to vary between values of t of $-1°$ C. to $+1°$ C. according to the incubation temperature and the period of storage. To adjust for these variations in the operation of this test in a practical scheme of temperature compensation, a series of tables would be required—one for every combination of storage and incubation conditions used—and to draw up such tables the present data are inadequate.

It has already been emphasized that the $r(t)$ values derived from the results of the methylene-blue test at 37·5° C. (Exp. 4) may not be reliable. In view of the small number of results available in some cases, and of the differences between centres, it would be dangerous to attempt to build on them.

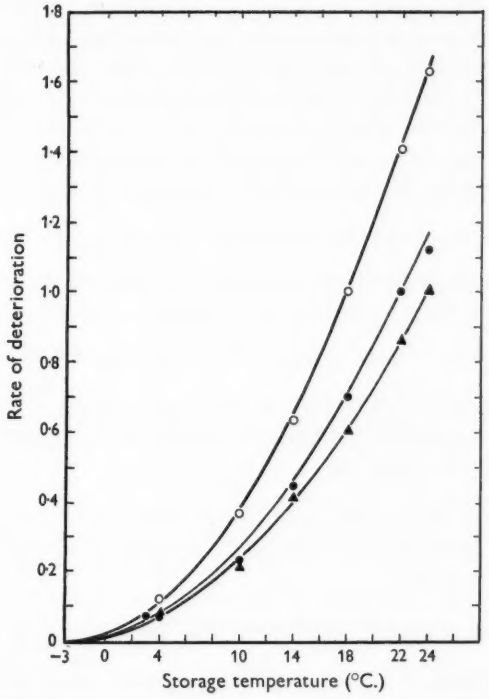

Fig. 4. Comparison of the observed $r(t)$ with the theoretical $R(t)$ rate of deterioration during controlled storage. ○, $r(t)$ for 18° C. incubation temp.; ●, $r(t)$ for 22° C. incubation temp.; ▲, $r(t)$ for 24° C. incubation temp. Value given is mean for K.Q. tests (Table 5). $R(t)$ curve given by full line.

COMPARISON OF CALCULATED WITH OBSERVED RESULTS

In the first (p. 45) and second (p. 46) experiments, for each sample, one subsample was stored at shade atmospheric temperature (2) in addition to the subsamples stored at the controlled temperatures. The times to end-point of the subsamples stored at shade atmospheric temperature have been compensated for the period of storage at this temperature. Thus, if a subsample reached its end-point with C.O.B. or A.P.T. in 21 hr. at 22° C. after 9 hr. storage at a mean shade atmospheric temperature of 19·3° C., then the time to end-

point of that subsample, had it been at 22° C. from the beginning, is calculated as follows:

$$\text{Equivalent total time at } 22° \text{ C.} = 21 + 9\left(\frac{19{\cdot}3+3}{22+3}\right)^2 = 28{\cdot}16 \text{ hr.}$$

The method has been used to adjust the results for the subsamples stored at shade atmospheric temperature to give the equivalent times to end-point at 18 and 22° C. The distribution of the differences between the calculated and the observed times for the corresponding subsamples stored continuously at 18° (Exp. 1) or 22° C. (Exp. 2) are given in Table 6.

A POSSIBLE GRADING SCHEME FOR MILK

The formula derived may be used to devise a grading scheme for milk, tested by C.O.B. or A.P.T., which will allow compensation for any pre-test storage. For example, assume that the standard to be applied to all milk samples is a keeping quality at 22° C., using C.O.B. as the end-point, of not less than 24 hr. from the time of collection of milk at farms; and, further, that milk is collected at farms at 9 a.m., but the incubation of samples at 22° C. is not possible until 4 p.m. It is required to make allowance for the 7 hr. interval between collection and examination during which the samples are assumed to be at mean shade atmospheric temperature. This 7 hr. is equivalent to $7 \times \left(\frac{t+3}{22+3}\right)^2$ hr. at 22° C., where t is the mean shade atmospheric temperature at which the samples are stored. To comply with the standard, therefore, the samples must give a negative result with the C.O.B. test applied after $\left[24 - 7\left(\frac{t+3}{25}\right)^2\right]$ hr. of incubation at 22° C., starting at 4 p.m. If the mean shade atmospheric temperature (t) between 9 a.m. and 4 p.m. on the day of sampling is 12° C., samples should give a negative result after incubation at 22° C. for (24 − 2·52) hr., i.e. at 1.30 p.m. on the day after sampling.

Table 7. *Testing times, for samples stored between* 9 *a.m. and* 4 *p.m. at shade atmospheric temperature and subsequently incubated at* 22° C., *to adjust the results to a keeping quality standard of* 24 *hr. at* 22° C.

Storage time on day of sampling	Mean shade atmospheric temp. between 9 a.m. and 4 p.m. in the range		Incubation at 22° C. from 4 p.m. on day of sampling to the following times on day after sampling
	° C.	° F.	
9 a.m. to 4 p.m.	Under 6·4	Under 43·6	3.30 p.m.
	6·4–10·3	43·6–50·6	2.30 p.m.
	10·4–13·3	50·7–56·0	1.30 p.m.
	13·4–15·8	56·1–60·5	12.30 p.m.
	15·9–18·0	60·6–64·5	11.30 a.m.
	18·1–20·0	64·6–68·2	10·30 a.m.
	20·1–21·9	68·3–71·5	9.30 a.m.
	22·0–23·6	71·6–74·6	8·30 a.m.

Table 7 gives, for this particular standard of keeping quality, the appropriate testing times at intervals of 1 hr. for samples taken on days when the mean atmospheric shade temperature between 9 a.m. and 4 p.m. is 23·6° C. or less.

A similar table could be derived to give appropriate testing times for any other keeping quality standard that might be required.

RELATIONSHIP BETWEEN KEEPING QUALITIES AT DIFFERENT INCUBATION TEMPERATURES

When keeping quality is estimated by incubation at one temperature it will often be necessary to know its equivalent at another temperature of incubation. For instance, if a milk has a keeping quality of 24 hr. at 18° C. how long should it keep when it is incubated continuously at 22° C.?

From the formula, each hour of storage at 18° C. before transfer to incubation at 22° C., is equivalent to $\left(\frac{21}{25}\right)^2 = 0{\cdot}706$ hr. at 22° C. It follows that a keeping quality of 24 hr. at 18° C. is therefore equivalent to 16·9 hr. at 22° C.

Direct information on the relationship between keeping quality results at 18 and 22° C. was obtained in the second and third experiments. In Exp. 2 the results for special subsamples, incubated continuously at 18° C. until end-point, can be compared with those for subsamples stored continously at the incubation temperature of 22° C. In Exp. 3 the results with subsamples stored continuously at the incubation temperatures 18 and 22° C. can be compared.

Using the ordinary methods of linear regression the relationship between the time to C.O.B. at 22° C., y, and the time to C.O.B. at 18° C., x, is as follows:

$$\text{from Exp. 2} \quad y = 3{\cdot}645 + 0{\cdot}615x;$$

$$\text{from Exp. 3} \quad y = -0{\cdot}350 + 0{\cdot}700x.$$

The relationship $y = 0{\cdot}706x$ obtained from the temperature compensation formula agrees very closely with the regression obtained from Exp. 3. The regression from Exp. 2 is somewhat different; the value of y when $x = 0$ is 3·645, and consequently the slope of the regression line is less than 0·706, the two lines crossing near the mean value of x and y. That, in the Exp. 2 regression equation, there is a high value for the constant term suggests that a curved regression line may give a better fit since physically it would be expected that $y = 0$ when $x = 0$. The regression $y = -0{\cdot}075 + 0{\cdot}809x - 0{\cdot}002x^2$ is an improvement. It may be noted that a curvilinear relationship would be expected if the temperature compensation curves were not parallel (i.e. if $B \neq 1$).

The slope 0·706 is based on an assumption of parallelism between the temperature compensation curves and gives the best estimate of the relationship between y and x on that assumption.

The keeping quality, y hr., at an incubation temperature T_1° C. equivalent to a keeping quality of x hr. at T_2° C. may therefore be derived from the expression

$$y = x\left(\frac{T_2+3}{T_1+3}\right)^2.$$

From this formula information can be obtained about the relative keeping quality of samples incubated at different temperatures. Thus, if a milk sample has a keeping quality of 60 hr. at 15·5° C. (T_1° C.) its keeping quality at 20° C. (T_2° C.) would be given by

$$60 = x\left(\frac{23}{18{\cdot}5}\right)^2,$$

whence the keeping quality x at 20° C. is $\frac{60}{1{\cdot}545} = 38{\cdot}8$ hr.

SUMMARY AND CONCLUSIONS

1. The experiments described in this part of the report were designed to obtain information about the effect of time and temperature of pre-test storage on test results and, in particular, about the possibility of a practical method of compensating test results to eliminate or minimize their dependence on the time and temperature of pre-test storage.

2. Four separate experiments were planned and carried out in three widely separated areas in England and Wales. Samples of morning milk, brought to the laboratory with the minimum of delay, were divided into a series of subsamples which were subsequently stored at controlled temperatures ranging from 3 to 24° C., and at shade atmospheric temperature, for periods ranging from 7 to 30 hr. before examination by one or more of the following tests: keeping quality at 18, 22 and 24° C. using clot-on-boiling (C.O.B.), and precipitation with 68% alcohol (A.P.T.) or 50% alcohol; methylene-blue test at 37·5° C. and at 18, 22 or 24° C. The results for the different tests were the times to end-point *after* transfer from the storage to the incubation baths.

3. The curves, known as temperature compensation curves, obtained for individual samples by plotting the results of tests after storage at the different temperatures, indicate the general conclusions to be drawn from the data.

Inspection of the curves and statistical analysis of the data in three experiments show that the keeping quality curves conform to a general pattern; they are virtually parallel and there is satisfactory differentiation between samples with all pre-test storage conditions investigated. Temperature compensation may therefore be applied to keeping quality results. This means that, with a relatively small error, the results to be expected after storage at one temperature may be calculated from the results obtained after storage at any other temperature.

The curves for the methylene-blue test results at 37·5° C. converge sharply after pre-test storage at the higher temperatures for 22 hr. After storage at 22° C., identical or nearly identical results were obtained from samples that gave widely differing results after storage at lower temperatures. The inference to be drawn from the diagrams is that after such a long storage period the milks divide into good and bad and consequently two different types of curves are met with. Any forecast from the results after storage at one temperature of the results after storage at any other temperature will, therefore, lead to large errors with individual samples. Temperature compensation of the results of the methylene-blue test at 37·5° C. after pre-test storage of samples for a period of 22 hr. is therefore not justified. For methylene blue at 37·5° C. the curves must still be regarded as convergent after a storage period of 8 hr., though the degree of convergence has decreased so much that individual results do not become confused in the higher temperature range. It appears that comparable curves are obtained for both good and bad milks. It may be that compensation is possible with a short period of storage, say 8 hr., for the temperatures under review, but the experiment is too small to make positive statements or calculate a scale.

4. For each test the means of the times to end-point, after every combination of time and temperature of pre-test storage, have been used to calculate $r(t)$ values. These $r(t)$ values represent the ratio of the rate of deterioration during 1 hr. pre-test storage at temperature t° C. to that of deterioration during 1 hr at the incubation temperature T° C.

The values of $r(t)$ obtained in the different experiments for the C.O.B. and A.P.T. tests

are in good agreement; they vary with the incubation temperature and with the pre-test storage temperature but are not affected by the duration of pre-test storage. With pre-test storage at the higher temperatures the values of $r(t)$ for the methylene-blue test at 18, 22 and 24° C. are in good agreement with those of C.O.B. and A.P.T., but they tend to be lower after pre-test storage at the lower temperatures. With storage at 4° C. the $r(t)$ values for the methylene-blue test are consistently negative, which means that storage at this temperature increases the time to end-point with this test. When the methylene-blue tubes are not inverted regularly, negative values are also obtained with storage at 10° C.

5. The experimental values of $r(t)$ for C.O.B. and A.P.T. are fitted by the curve represented by the formula $R(t)=\left(\frac{t+3}{T+3}\right)^2$, from which it is concluded that each hour of storage at t° C. may be regarded as equivalent to $\left(\frac{t+3}{T+3}\right)^2$ hr. at T° C.

The results for the methylene-blue test at 18, 22 and 24° C. do not fit this formula.

No conclusive evidence is available regarding methylene blue at 37·5° C. The $r(t)$ values are based on few results and this may account for apparently wide differences between centres. No attempt is made to draw conclusions which might prove invalid if the experiment were repeated on a larger scale.

6. The formula has been used to calculate the results to be expected after pre-test storage at the incubation temperature from those obtained after pre-test storage at shade atmospheric temperature. The actual and calculated results are in good agreement.

7. An example is given of the use of the formula to devise a grading scheme in which the time of testing is varied according to the pre-test storage temperature to ensure compliance with a stipulated standard.

8. An example is also given of the use of the formula with A.P.T. or C.O.B. for the calculation of the time to end-point of a sample after incubation at any temperature from the time to end-point of the same sample incubated at a given temperature. The theoretical values so obtained are shown to be in good agreement with experimental results for duplicate portions of the same samples tested at 18 and 22° C.

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436. THE ESTIMATION OF CITRIC ACID IN DAIRY PRODUCTS

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(With 1 Figure)

INTRODUCTION

The determination of citric acid in milk and in milk products has always presented many difficulties. An extensive literature exists on the determination of citric acid in fruit juices and biological liquids, but until lately no rapid method of determining citric acid in milk and milk products has been available. The citrates exert considerable influence on the colloidal systems of milk and its products. They also provide material for the aroma-yielding substances produced in the course of milk fermentation brought about by micro-organisms like *Leuconostoc citrovorum* and *Leuc. paracitrovorum*.

In the course of our work the need arose for a rapid and accurate micro determination. The classical method by Stahre (1), in which the citric acid is determined as pentabromo-acetone, and which has hitherto been used for want of a better one, is based on the oxidation by $KMnO_4$ to acetone dicarboxylic acid, which is in turn converted into pentabromoacetone by treatment with bromine. The method, as modified by Kometiani (2), Lampitt & Rooke (3) and many others, has several drawbacks, of which the main are: (i) it is time-consuming (2–3 days) and unsuitable for routine testing; (ii) the precipitate is often obtained as oil, and not in the crystalline form; (iii) the precipitate is relatively soluble; (iv) the precipitate is volatile when wet (see discussion by Allen (4)). In the modification by Deysher & Holm (5) the time of the determination is extended by a further 24 hr. Modern colorimetric methods, adapted to biological liquids and based on pentabromoacetone, *are unsuitable* for milk and its products as shown by Sjöström & Emilsson (6).

Two modern methods have appeared to us to deserve consideration: (i) the Cerate Oxidimetry method by Heinemann (7); and (ii) the method of Saffran & Denstedt (8) for blood. The former method involves working at low temperatures, which is difficult in Israel, where the mean temperature in summer is 30° C.; we have accordingly chosen the latter, and succeeded in developing, with certain modifications, a method suited to our purposes.

The method is based on the colour reaction of Furth-Hermann (9), in which citric acid heated with a mixture of pyridine and acetic anhydride, develops a permanent yellow coloration, which may be measured photometrically. The test is simple and rapid, and may be used to determine 50–450 μg. anhydrous citric acid. Smaller quantities, as low as 10 μg., may also be determined, although with less accuracy. The lowest concentration of citric acid which can be determined by this method is 0·01 %.

* This work was carried out within the framework of an extensive research programme on 'Products made from fresh and reconstituted milk and their amelioration', with the grant of the Scientific Council of the Prime Minister's Office, Government of Israel.

EXPERIMENTAL

Reagents

(1) A 10% solution of trichloroacetic acid (CCl_3COOH A.R.).

(2) Dry acetic anhydride, C.P.

(3) Pyridine (for chemical use, Eastman Kodak Co.).

(4) Analytical pure citric acid ($H_3C_6H_5O_7H_2O$), a freshly prepared solution of 10 mg./ml. However, solutions may be preserved for longer periods under toluene. It is diluted to the desired concentration. It should not contain H_2SO_4 (see below).

Apparatus

(1) Pipettes of 1 ml., graduated in 0·01 ml. divisions (retested).

(2) Graduated colorimetric tubes (we used ordinary Pyrex test-tubes, 14 mm. in diameter, previously tested for equal transmission of light).

(3) Photoelectric colorimeter (we used a Photoelectric Lumetron Colorimeter Type 402-E).

Procedure

To an accurately measured quantity of milk (or any other product), an equal volume of the 10% trichloroacetic acid solution is added. The mixture is shaken and left for about 2 hr., to ensure a better precipitation of the proteins. It is then filtered, and the first part of the filtrate discarded. Where speed is essential this waiting may be dispensed with.

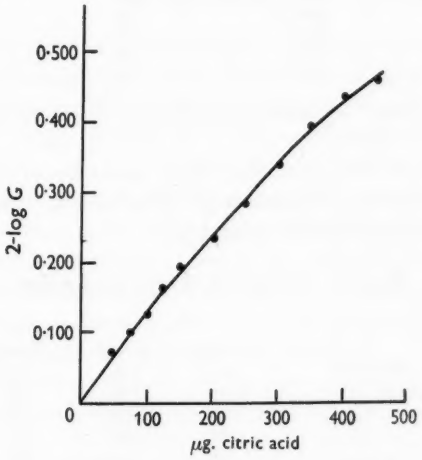

Fig. 1. A typical calibration curve (G = percentage light transmission).

Exactly 0·2 ml. of the filtrate are pipetted into the dry colorimeter tubes, and 7 ml. of acetic anhydride added from a burette. The tubes are then firmly closed with rubber stoppers and put into a constant temperature water-bath, at 60° ± 1° C.

After 10 min., the tubes are taken out, 1 ml. of pyridine is added to each, and they are immediately stoppered. They are then put back into the water-bath for 40 min. more, during which time they are shaken every 5–8 min. After that the tubes are put into an ice

water-bath for 5 min. Finally they are taken out, wiped dry, and after 10–15 min. the colour is read in the Lumetron Colorimeter with the 420 mμ filter. It is recommended to run three parallel determinations of each sample at a time.

Two blanks starting with 0·2 ml. portions of a 5% solution of trichloroacetic acid are run simultaneously in each series of tests, and used to set the colorimeter.

A calibration curve is prepared with solutions of 50–150 μg. citric acid in 0·2 ml. of 5% trichloroacetic acid. A typical curve is given in Fig. 1.

With every new batch of reagents, a new calibration curve should be prepared.

In the determination of citric acid in cottage cheese, 2·5 g. of finely divided cheese in 10 ml. water at 50° C. are vigorously shaken and an aliquot taken for analysis.

With a little practice, and under suitable conditions, as many as sixty tests may be carried out in 1 day by a single worker.

RESULTS

As an example, the results of nine parallel determinations on a milk sample are shown in Table 1. It can be seen from the table that the accuracy is approximately 2%.

Table 1. *The reproducibility of the determination*

Serial no. of test (n)	Light transmission (%)	Citric acid (μg.)	Deviation from average (d)
1	59·0	188	0
2	59·5	184	−4
3	58·5	192	+4
4	59·5	184	−4
5	59·0	188	0
6	58·5	192	+4
7	59·0	188	0
8	58·5	192	+4
9	59·5	184	−4

Average 188 μg. Standard deviation $\pm\sqrt{\frac{\Sigma d^2}{n-1}} = \pm 3{\cdot}5$ ($\pm 1{\cdot}9$%).

The method was then checked by adding known quantities of citric acid to the product tested and testing the recovery. Results are given in Table 2. (Each result is the mean of three, and deviations were usually less than 4%.)

Table 2. *Recoveries of citric acid added*

No.	Description of sample	In sample (μg.)	Added (μg.)	Found: Total (μg.)	Found: Added (μg.)	Recovery found (%)
1	Pasteurized whole milk	160	60	220	60	100
		160	120	280	120	100
		160	180	342	182	101
2	Sterilized milk	140	167	315	175	105
3	Milk reconstituted from skim milk powder	196	60	258	62	103
		196	180	385	189	105
4	Naturally soured milk	15	120	140	125	104
5	A culture of *Leuc. citrovorum* in skim milk	15	150	160	145	97
6	Lebben	20	200	212	192	96

The presence of lactose, phosphoric acid or lactic acid does not interfere with the determination. Heinemann (7) rules out the possibility of a method based on pyridine-

acetanhydride as the presence of Cl ions makes such a method unsatisfactory. We therefore conducted a series of experiments in order to estimate citric acid in the presence of Cl, bearing in mind the fact that milk products usually contain some Cl ions. However, we found that their presence does not interfere with the determination.

Results of determination of citric acid on various milk products are given in Table 3. We have also determined the percentage of citric acid in eight samples of whole milk and have found a mean value of 0·193 and extreme values of 0·160–0·245. The figures agree with the data for milk in the literature.

Table 3. *Citric acid concentration in various milk products*

Description	Sample no.	Citric acid (%)	Remarks
Skim milk powder	1	2·52	In dry matter
Skim milk powder	2	1·91	In dry matter
Lebben, manufactured by Tnuva from reconstituted milk	1	0·015	—
	2	0·028	—
Cottage cheese	1	0·03	In dry matter
Cottage cheese	2	0·03	In dry matter
Mixed whole milk	1	0·194	—
	2	0·196	—
	3	0·180	—
	4	0·188	—
	5	0·245	—
	6	0·216	—
	7	0·167	—
	8	0·160	—

DISCUSSION

It is noted in the literature (8,9) that water has a great effect on the uniformity of colour development. We did not obtain good results with 1·0 ml. of the solution tested, and therefore we reduced the volume to 0·2 ml. Much better results were then obtained.

It is clear from Fig. 1 that the intensity of colour deviates from Beer's law (compare also (8)).

It is recommended not to use citric acid solutions that are preserved with sulphuric acid against fungal growth. When acetic anhydride is added to such solutions, and the tubes are heated in the water-bath, a violent reaction takes place. The stoppers are thrown out and a mixture of hot acetic acid and anhydride is ejected from the tubes. It can be assumed that this reaction is caused by the catalysing effect of the hydrogen ions from the sulphuric acid, as the same phenomenon was also observed with HCl.

Standard conditions must be observed during work, otherwise results are not accurate. Changing the temperature of the water-bath or not shaking the tubes in it causes considerable errors.

The results given in Tables 1 and 2 show that the accuracy of the method is better than $\pm 5\%$, usually $\pm 3\%$. There are sources of inaccuracy which make better results impossible. Of these the most important are:

(1) The measurement of the volume of the solution tested. In checking the accuracy of delivery 0·2 ml. of water from the 1 ml. graduated pipette, we found deviations up to 2%.

(2) The sensitivity of the colorimeter. In the one we used we could read light transmissions to an accuracy of 0·5% only. This is called the absolute photometric error (10).

This introduced an error of about 3·5% ($\pm 1{\cdot}8\%$) in the determination of 50–200 μg.

citric acid and a lower error in larger quantities. This error may also be calculated according to Ringbaum's formula given by Ayres(10):

$$\frac{\%\text{ relative analysis error}}{1\%\text{ absolute photometric error}} = \frac{230\,d\log c}{dI},$$

where c is the quantity of citric acid and I the percentage of light absorption $(100-G)$.

In our case, where the absolute photometric error is 0·5%, the analysis error introduced by the colorimeter will be $\frac{1}{2}\cdot\frac{230d\log c}{dI}$.

Table 4 gives the results of this calculation. The values of I are according to Fig. 1.

Table 4. *Evaluation of error*

Citric acid range μg. (c)	Analysis error per 0·5% photometric error (%)
10–50	7·5
50–100	3·7
100–200	3·4
200–400	1·6

The optimal range is therefore 200–400 μg., which is very suitable for estimations in milk.

SUMMARY

A rapid method for the colorimetric analysis of micro quantities, of citric acid beginning at concentrations of 0·01% of citric acid in milk and in dairy products has been described. The analysis is based on the yellow colour which develops upon reacting citric acid with pyridine in a solution of acetic anhydride, as applied by Saffran & Denstedt for the estimation of citric acid in blood. The accuracy of the method in the ranges normally used in the estimation of citric acid in milk is approximately ±3%. Examples of determinations in milk and dairy products are given.

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437. COPPER AND IRON IN DAIRY PRODUCE. IMPROVED METHODS OF ANALYSIS

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CONTENTS

	PAGE
Introduction	77
Cream supplies	78
Experimental	79
(1) Outline of methods	79
(2) Preparation of reagents	80
(3) Filtration method	81
(4) Wet-ashing method	83
(5) Blanks and standards	83
(6) Solvent recovery	84
Discussion	85
Results	87
Further notes	92
Summary	93
References	93

INTRODUCTION

Nearly sixty years ago Weigmann (1) first showed that minute traces of iron might help to produce off-flavours in butter, but even thirty years ago the undesirable effects of metallic contamination of dairy products were hardly realized. This is clear from Sommer & Smit's (2) paper, 'The fishy flavour in butter', in which the literature is fully reviewed. These authors mention that in 1914, Rogers (3) pointed out that as little as one part of iron per million of cream had a marked effect on the flavour of the resulting butter. Within the next few years other workers, including Hunziker (4), began the first systematic experiments to show that minute traces of copper and iron tend to catalyse oxidative changes within the fat, leading to such defects as 'fishiness' and 'tallowiness' in dairy produce, but even then results were obtained chiefly by the addition of known small amounts of metals and observing the flavour that developed on storage. To ascertain the precise amount of metallic contamination in defective produce, reliable methods of analysis have been slowly developed by much patient work in the last twenty-five years.

Supplee & Bellis (5) appear to have been the first to attempt to estimate by analysis the amount of copper present in milk, using a dry-ashing technique. In 1927, one of the authors (G.M.M.) observed Davies' first attempts to apply a dry-ashing method to butter (6). However, both these chemists obtained results that were rather high. Dry-ashing as improved by MacFarlane (7) has been applied to butter in the authors' laboratory, but wet-ashing as developed by Williams (8) has been found preferable, especially if modified as suggested by Koppejan & Van der Burg (9) and others. Recent workers who have applied wet-ashing to butter are Hussong & Hammer (11), and McDowell (12). Ashing methods, however, evolve large amounts of fumes and are usually tedious and time-consuming, so that comparatively small numbers of samples can be dealt with, while large amounts of chemicals are required.

The study of blood chemistry, along with a knowledge of the chemistry of the fat and the proteins of milk, led to the idea that it should be possible to extract the metals with acid from the compounds present in the butter, and after removal of the fat, to filter off the protein materials precipitated by trichloroacetic and tungstic acids. A similar method was suggested by Tompsett (15*c*) for the estimation of iron in milk, while Tompsett's method for copper in blood (15*b*) was applied by McIlroy (10) to the determination of copper in milk. Thus methods were evolved in the authors' laboratory for the estimation of copper and iron in butter (13), and also of copper in cream (14) by a filtration procedure which has been checked with an improved wet-ashing technique. The present paper describes the extension of this work to include the estimation of iron in cream, and introduces modifications needed to provide a combined method in which both copper and iron are estimated on the same sample. Both the filtration and the wet-ashing methods have been successfully applied to buttermilk. For cheese also, the wet-ashing method has been used, and it may be preferable for milk.

CREAM SUPPLIES

The name 'creamery' butter is applied in New Zealand to what is made in a 'creamery' or butter factory, from cream separated from fresh milk, and this amounts to over 97% of the total butter output. Nearly all the cream is 'home-separated', i.e. skimmed on the dairy farm immediately after milking, but a small proportion is separated at skimming stations to which the milk is delivered daily, so that the skim-milk can be used for making dried milk, and casein. In the principal dairying districts the cream is collected daily from the farms, except in the autumn and winter, when collection may be every second or third day. Where dairying is a minor side-line, the amount of cream is usually small and may be sent away from the farm only once a week. Such cream is usually sour and the acid developed has ample time to dissolve iron from any portions of the cream-can whence the tinning has become worn. By contrast, cream which is delivered daily has little opportunity for gathering up iron unless the farm utensils are rusty.

In cheese factories the whey from the vats contains from 5 to 10% of the fat present in the original milk, and by passing this whey through a separator, most of the fat can be saved to make whey butter. The acid developed in the vat enables the whey to dissolve more iron, so whey cream and butter have a higher iron content. The protein in whey cream is chiefly lactalbumin, while the mineral constituents are also appreciably different from those in ordinary cream. Whey cream usually has a fat content of about 35%, and to check further development of acid it is pasteurized immediately after separation by standing the can in a tub of water at about 200° F. The whey cream from neighbouring cheese factories is usually gathered to a central butter factory where it is neutralized, flash-pasteurized, and churned. Whey butter is thus manufactured in much the same way as creamery butter, and in the smaller units is made two or three times a week. The churn for whey butter may not be used for creamery butter, lest the latter be spoiled by the characteristic flavour of whey cream. When freshly made, good whey butter is quite satisfactory for table purposes and much is so used by suppliers of New Zealand cheese factories, but it may be less satisfactory after some weeks' storage. At the time of grading within a few days of manufacture, defects in good creamery butter due to metallic contamination are rarely noticeable, and even after a period of cold storage such defects may not be as obvious as in the case of whey butter, but the flavour may be merely flat or 'fatty', or lacking in the character expected of really good butter.

In the manufacture of both creamery and whey butter, copper contamination gets into the cream from untinned copper and brass fittings associated with the factory equipment, especially the receiving vat, cold vat or the churn. Iron contamination seldom occurs within the actual butter-making plant; the commonest source there is the wash-water which often has a higher iron content than is desirable, due to rust from the chilling coils. The main source of iron is rusty cans, but if these and other farm utensils are in good order, butter made from fresh cream should have a low iron content. Although cream naturally contains more iron than copper, the latter is well known to be more active in catalysing fat auto-oxidation, so that much less copper may initiate deterioration of the butter.

Metallic contamination has been checked by the increasing use of stainless steel, but during the war difficulties arose in obtaining supplies for replacing old equipment, and also for retinning what could not be replaced. The shortage of edible fats in England in recent years compelled the sale for table use of whey butter, which in the past was used chiefly by bakers, etc., so special efforts have been made to improve its quality.

EXPERIMENTAL

(1) *Outline of methods*

The filtration method for butter depends essentially on the quantitative extraction of the metals with acid into the aqueous phase, followed by precipitation of protein and its degradation products. After removal first of the fat and then of the protein precipitate by filtration, colorimetric estimation of copper and iron can be carried out on aliquot portions of the acid filtrate. In his investigations upon blood and other biological material, Tompsett (15*b*, *c*, 16) has shown that when trichloroacetic acid is used to precipitate the proteins, all the copper and the ferrous iron pass into the filtrate. Prior to filtration in the case of butter, the fat must be separated and drawn off by suction, while in the case of cream a necessary preliminary consists of churning the weighed sample (as in laboratory butter-making experiments) in order to break the fat-in-water emulsion. For buttermilk and whey samples, protein precipitation is similar to that for butter and cream, but centrifuging and removal of the fat are unnecessary. Concentrated nitric and hydrochloric acids have been found equally efficient for the extraction of copper, but excess may cause fading or interference if the iron is to be finally determined as the thiocyanate complex. For cream, the addition of acid prior to the chilling and churning of the sample, facilitates both the extraction of the metals and the churning process.

According to Tompsett (15*a*, *c*) protein complexes containing ferric iron are much more stable than those of ferrous iron, and for this reason, to ensure the extraction of all the iron, a strong reducing agent must be used in conjunction with the hydrochloric acid. The most suitable reducing agent is sodium hyposulphite ($Na_2S_2O_4$), but the samples should be kept warm overnight to complete the extraction of iron, and also to decompose the excess hyposulphite before filtration, so that no sulphur is subsequently deposited in the filtrate. Hyposulphite is preferable to *p*-hydroxy-phenyl-glycine (18); and to thioglycollic acid (12) which may produce insoluble copper sulphide.

The wet-ashing method described in the earlier papers has been extended to allow the estimation of both copper and iron on the same sample by taking care to avoid excess of sulphuric acid and by attention to other details referred to later.

(2) *Preparation of reagents*

Due care must be taken at all stages to avoid contamination of reagents, samples, etc. All glassware after thorough cleaning is rinsed with dilute nitric acid (5%), and then with distilled water. Rubber stoppers should be soaked in dilute nitric acid for a couple of hours before use. Glass-distilled water is desirable, but water from a large stoneware still (Gallenkamp, No. 4483) is also satisfactory.

Acids of A.R. quality are preferable but they should be tested for metals. Commercial hydrochloric and nitric acids may be purified by distilling in an all-glass still. Before distilling hydrochloric acid it should be diluted to sp.gr. 1·12, and the distillate adjusted to be 6 N. Trichloroacetic acid should be distilled *in vacuo*, and 20% solution made as required.

A fresh 5% solution of sodium hyposulphite ($Na_2S_2O_4$) should be made every 4 weeks. If necessary, partial purification of this may be effected by the addition of a few drops of ammonia, followed by filtration, after which the solution is re-acidified with the equivalent drops of hydrochloric acid. Another method of purifying this reagent has been described by Hill (17), but has not been needed in the present work.

To purify the 10% solution of sodium tungstate it should be warmed prior to adding a few milligrams of *solid* carbamate reagent and shaking several times with amyl acetate in a separating funnel.

Hot water is used to make the 50% solution of ammonium citrate which may need to be filtered. After making alkaline to phenolphthalein with 0·88 ammonia, a few milligrams of solid carbamate reagent are added, followed by several extractions of the warm solution (50–60° C.) with amyl acetate in a separating funnel. The resulting solution should be re-acidified with the equivalent amount of hydrochloric acid. Eden & Green (20) recommend this salt instead of sodium citrate.

Ammonium thiocyanate seems preferable to the potassium salt, especially as it can be readily purified (24) and stabilized. The iron blank value has thus been appreciably reduced. To purify the 30% solution of this salt, make it just acid with a few drops of hydrochloric acid and warm to 50–60° C. After two or three extractions with amyl alcohol to remove traces of iron, a few drops of ammonia are added to make the solution alkaline. This solution is then stable if kept in the dark. This and other purified reagents are best stored in small bottles to check recontamination.

The carbamate reagent for copper is made by dissolving 0·2 g. of sodium diethyl-dithiocarbamate in 90 ml. of water and 10 ml. of 0·88 ammonia. This should be stored in a dark bottle and renewed after 2 or 3 weeks.

The purification of iso-amyl alcohol and iso-amyl acetate is described in detail later. Other reagents such as ammonia, hydrogen peroxide, phenolphthalein solution (1% in redistilled alcohol) should be tested for metallic contamination in amounts likely to give rise to errors.

Standard copper and iron solutions are made up so that 1 ml. = 1 mg. of metal. For this strength dissolve 0·3928 g. of A.R. crystalline copper sulphate in water (with two or three drops of sulphuric acid to check hydrolysis) and make up to 100 ml. For iron dissolve 0·864 g. of A.R. ferric ammonium sulphate, $FeNH_4(SO_4)_2.12H_2O$, in distilled water containing 10 ml. of 6 N-hydrochloric acid, and make up to 100 ml. From these solutions further dilutions are prepared so that 1 ml. = 0·01 mg., and 1 ml. = 0·001 mg. of metal.

Especially in the case of iron the lowest dilution should be prepared frequently, as iron often deposits on the glass from very dilute solutions.

In the past, Whatman nos. 40, 42, and 50 filter-papers have been used, but all need washing with 5% nitric acid in the funnel just before use. If a cheaper quality, such as no. 4, be used, more thorough acid washing is needed in a deep Petri dish or Buchner funnel (with rubber stopper below). If batches of papers which have been acid washed are not being used at once they should be given a final rinse with dilute ammonia. No. 50 papers can be used two or three times if promptly and carefully cleaned, but slow filtration and other difficulties may then occur.

(3) *Filtration method*

If a large piece of butter is to be divided up, it should be placed in a sealed glass jar and left for some hours in an incubator at 20–22° C., and it can then be well mixed without melting. Alternatively, suitably prepared tubes may be used for butter samples. Cream and buttermilk samples may be preserved with 1 ml. of A.R. formaldehyde per half-pint, and the bottles kept in a refrigerator.

Duplicate 25 g. portions of the samples are weighed into suitable 100 ml. centrifuge tubes. As previously described (13), the butter is removed from glass sample tubes by gentle warming and blowing through a small hole, while cream samples are warmed in the bottles to ensure thorough mixing before weighing out (14). To each centrifuge tube with butter 4–8 ml. of hot water are added when placing in a bath at 45–50° C. When the butter has melted and the curd-serum layer separated, 4 ml. of 6 N-hydrochloric acid are added and each sample is well mixed. Buttermilk and whey samples require 5–6 ml. of hydrochloric acid when placed in the water-bath. On account of the greater amount of protein and hence greater bulk of precipitate, for buttermilk an L-shaped glass rod may be needed in each tube to break up the curdy material and thus ensure the extraction of the metals. In the case of cream, after the addition of 5–6 ml. of 6 N-hydrochloric acid, the centrifuge tube is closed by a suitable acid-washed rubber stopper and cooled for an hour or more in ice-water to harden the fat prior to churning. The tubes are then placed in a motor-driven shaking-machine consisting of a box (fitted to hold twelve stoppered centrifuge tubes placed lengthwise) which is subjected to a rapid to-and-fro-motion (Baird and Tatlock, Ltd., cat. no. WDC 2950). By such treatment the fat in the cream sample is transformed to a round lump of butter in 20–30 min.* After churning and before opening the centrifuge tube, the outside of the rubber stopper is washed carefully with distilled water and the inside of the stopper is rinsed with distilled water into the appropriate tube. The samples are then melted by placing in the water-bath for about 20 min.

To all samples, after heating with acid as already described, 2·5 ml. of 5% hyposulphite solution is added, and the samples are kept at about 45–50° C. with frequent gentle shaking, for a further hour. A short time, 2 hr., has proved insufficient to extract all the iron, so it is preferable to keep the samples at 37–40° C. overnight. Next morning the tubes are

* Cream samples vary appreciably in churnability. The optimum churning temperature is about 8–10° C., but owing to the peculiar physical properties of the fat globules, cooling to this temperature does not usually suffice. The best method is to cool the samples in ice-water for an hour or two before churning. Prolonged cooling (e.g. overnight at 3–5° C.) may, however, make the samples very difficult to churn. The fat then becomes too hard, and after failure to churn in such cases it has proved useful to hold the tube for a minute in hot water, 60° C. and then shake vigorously. Although some samples churn easily with a few minutes' shaking by hand, mechanical shaking is preferable.

warmed to at least 45° C. for a short time, prior to addition of the protein precipitant, 2·5 ml. of 20%, trichloroacetic acid solution for butter, cream and whey samples, or 5·0 ml. for buttermilk. The contents of the tubes are shaken as before and kept in the water-bath for a further 20–30 min. at 45–50° C. To complete the precipitation of protein residues, 10% sodium tungstate is next added, 0·6 ml. for butter and 1·5 ml. for cream, buttermilk and whey samples. During the next 20–30 min., the temperature is raised to at least 50° C. to ensure complete precipitation of the remaining sulphur and tungstic acid. At higher temperatures, the time specified for heating may be shortened, but loss of trichloroacetic acid due to hydrolysis may occur above 65° C. Butter and cream samples are then centrifuged at 1000–1500 r.p.m. for 5–10 min., after which most of the melted fat can be removed by suction without disturbing the watery layer. When the remaining fat has been completely solidified by standing in ice-water, the samples are filtered through wet filter-papers into suitable tubes, graduated at 50 ml. (stoppered measuring cylinders, or glass-stoppered test-tubes, B.S.S. no. 770, 1938, with stoppers C19). The filter-papers required are 11·0 cm. for butter, and 12·5 cm. for cream or buttermilk samples, depending on the size of the precipitate. Care should be taken at first to retain most of the precipitate in the centrifuge tube, where it can be mixed with successive washings by means of a glass rod, which is also useful to direct the washings round the upper portion of the paper. The washing of the residue in the centrifuge tube is done by three successive mixtures of 2 ml. of 5% nitric acid with 2 ml. of 5% trichloroacetic acid, allowing to drain well each time. Then a fine-tipped pipette is used to apply 3 ml. of 5% nitric acid around the upper edge of the filter-paper. A similar final wash with 3–5 ml. of water alone is usually practised, but may cause trouble, for reasons explained later. The filtrate is made up to exactly 50 ml. and, after mixing thoroughly, 10 ml. portions for iron test are pipetted into small acid-washed test-tubes. To oxidize any sulphite remaining, four drops (0·2 ml.) of 3% hydrogen peroxide are added to the iron portion. Peroxide should be avoided in the copper portion lest it cause fading of the carbamate colour, but more may be needed for the iron portion, where a slight excess may help to develop the full colour (22).

(*a*) *Copper.* To de-ionize iron, and also calcium, so as to avoid precipitation of phosphate, 4 ml. of 50% ammonium citrate are added, followed by one drop of phenolphthalein and sufficient 0·880 ammonia to make the solution definitely alkaline. After mixing, 4 ml. of 0·2% carbamate reagent and 4 ml. of amyl acetate are added and the tubes are warmed at 55–65° C. for 5–10 min. They are then stoppered and shaken vigorously thirty to forty times and allowed to stand until the acetate layer separates. Adequate warming and shaking are essential to secure complete extraction. The development of an emulsion at this stage may be due to various factors—incomplete protein precipitation, or to traces of protein being washed through the filter-paper. When quite cool the acetate layer may be removed.

(*b*) *Iron.* This portion is allowed to stand for at least 30 min. after the addition of hydrogen peroxide, after which 1 ml. of 30% ammonium thiocyanate and 4 ml. of amyl alcohol are added. In some cases a drop or two more of 3% peroxide may be needed at this stage (22). By inverting *gently* twenty to thirty times without warming, the red iron complex is completely extracted into the amyl alcohol, which usually clears within 20–30 min., and to avoid fading it should be removed from the aqueous layer without undue delay. If warmed before shaking, the amyl alcohol develops a cloudy condition which does not clear up readily.

(4) *Wet-ashing method*

The procedure followed is essentially that described for the estimation of copper in cream and buttermilk in an earlier paper (14). Briefly, 25 g. of the sample are placed in a large centrifuge tube with 10 ml. of nitric acid, and an L-shaped glass rod to break up the froth which develops when the tubes are warmed. The bath with the tubes is raised to boiling-point and the heating continued for 30–40 min. According to McDowell (12), the acid should be diluted with water (3 ml.) and the heating time shortened to avoid decomposing the fat. After cooling, the aqueous layer can be transferred (pipetted) to Kjeldahl flasks and the fat further washed repeatedly with a few ml. of nitric acid (1 part) diluted with hot water (3–4 parts). The combined extract is evaporated down and later 1 ml. of concentrated sulphuric acid added. Digestion is continued, adding more nitric acid as required till the liquid is almost colourless and sulphuric fumes begin to come off. Finally, a few drops of perhydrol and a little water are added and boiled off till sulphuric fumes reappear. Formerly when larger amounts of sulphuric acid were used, it was impossible to determine iron due to interference caused by the excess, also by nitrosyl sulphuric acid remaining after the digestion. In rare cases more sulphuric acid (0·5 or 1 ml.) may be required to replace what is used up, but if the amount remaining in the digest is less than 1 ml., and precautions are taken at the final stage for the decomposition of any nitrosyl sulphuric acid, there is no difficulty in determining iron on an aliquot portion. Following the digestion, the contents of the Kjeldahl flasks are transferred to extraction tubes graduated at 50 ml. and after rinsing out with 2–3 ml. of hot 6N-HCl, are made up to the exact volume. After mixing, 10 ml. for estimation of iron are pipetted into acid-washed test-tubes, to each of which is added one small drop of concentrated nitric acid. The remaining 40 ml. are used for the determination of copper, both procedures being the same as those described for the filtration method, under (*a*) copper and (*b*) iron.

(5) *Blanks and standards*

With each set of samples, two blanks containing all the reagents are included. The tubes with the filtration blanks may be stood for half an hour in hot water (about 80° C.) to precipitate sulphur and tungstic acid. Otherwise the blanks are treated in the same manner as the samples, then estimated separately and the average result subtracted from that obtained for each unknown.

Strictly speaking, the filtration standards should contain all the reagents as in the blanks, and should be treated in a similar manner. For the copper standards using carbamate and amyl acetate, careful trials have shown that trichloroacetic acid, sodium hyposulphite, and sodium tungstate may be omitted without any measureable effect on the results over the usual range up to say 1·0 p.p.m. For the iron standards, hyposulphite and tungstate may be omitted, but not trichloroacetic acid, of which the amount present slightly affects the development of the thiocyanate colour. With standard solutions containing hydrochloric and trichloroacetic acid, the pH values (determined electrically) of the solutions are very close to those of the corresponding sample solutions. For maximum development of thiocyanate colour, pH is critical (23). Loss of trichloroacetic acid during precipitation and heat treatment probably reduces the amount remaining in the filtrate so that in the case of butter and cream samples little if any trichloroacetic acid need be added to standards, especially if the iron values are less than about 3 p.p.m. But with buttermilk where more trichloroacetic acid is used, and also in samples with a high iron content, the standards

should have trichloroacetic acid included; half the amount added to the respective samples should suffice to give reliable comparisons. With these reservations, standards should contain only hydrochloric acid for filtration, or hydrochloric and sulphuric acids for wet-ashing; together with the same amounts of the appropriate reagents (citrate etc.) for copper, or for iron.

Thus when a 25 g. sample of butter, etc., is used, and the filtrate is made up to 50 ml., of which 40 ml. is used for the copper estimation, the standard (corresponding to 20 g. sample) for 0·10 p.p.m. requires 2 ml. of solution containing 0·001 mg. of copper per ml. After adding the same amount of hydrochloric acid as used for the unknowns (strictly speaking four-fifths the amount of acid, but the amount is not critical), the volume is made up to 40 ml. prior to adding the other reagents (citrate, etc.). If necessary less acetate, say 3 ml., may be used for both unknowns and standards to provide a smaller volume of deeper colour for the final comparisons.

The 10 ml. portion for the iron estimation represents 5 g. of sample, so that the standard for 1·0 p.p.m. requires 0·5 ml. of solution containing 0·01 mg. of iron per ml. After adding one-fifth of the amount of hydrochloric acid used for the unknowns (also 0·2 ml. of sulphuric acid for wet-ashing) the volume is made up to 10 ml. prior to adding the other reagents (one drop of nitric acid and four drops of 3% hydrogen peroxide). After shaking, the thiocyanate and 4 ml. of amyl alcohol are added and the colour extracted as for the unknowns. If desired, more or less alcohol may be used uniformly throughout. For very high values, besides adding trichloroacetic acid as already mentioned, 2 ml. of the coloured solvent may require to be diluted with 2 ml. or more of clear alcohol to enable readings to be made, but the results so obtained may not be quite accurate.

Apart from the influence of trichloroacetic acid upon the standards for iron, other trials showed that excess of nitric acid (five drops instead of one) did not affect the colour developed immediately, but later caused slight fading accompanied by the formation of a yellow precipitate. A similar excess of hydrochloric acid quickly produced fading and a yellow precipitate. When excess of hydrogen peroxide (ten drops) was used, the iron colour showed little if any change in 48 hr. For copper standards peroxide should be omitted lest it give rise to fading.

Both the amyl acetate and amyl alcohol layers are transferred by pipette operated by rubber teat to small vials. If the coloured extracts are not quite clear each tube should be dipped for a few moments in a conical flask full of warm water (50–60° C.) just prior to the final colorimetric estimation using the photo-electric absorptiometer* as described in an earlier paper (14). When the galvanometer readings are plotted against the values of the copper standards the curve is very nearly a straight line. But owing to deviation from Beer's law, in the case of iron the curve becomes increasingly steeper, with the result that for higher values (about 5 p.p.m. and above), values read from the graph become less accurate, because a slight difference in reading corresponds to an appreciable difference in the colour intensity.

(6) *Solvent recovery*

After the absorptiometric readings have been made, the amyl acetate and amyl alcohol are collected in separate residue bottles and may be purified by distillation. To

* A useful method of maintaining constant secondary battery voltage is to connect to the battery terminals the leads from a dry rectifier which derives its power through a transformer from the 230 V. a.c. mains. Fluctuations in mains voltage do not appear to affect the voltage supplied by the battery to the lamp. This arrangement is similar to the circuit given by Strafford (27), except that no barreter lamp is required.

check hydrolysis on heating, the acetate residue bottle should contain anhydrous sodium sulphate. The amyl acetate residues may be fractionated directly, that portion distilling between 136 and 140° C. being collected for re-use. Amyl alcohol residues are made alkaline by adding several grams of solid sodium hydroxide and then soda-lime until definitely alkaline, before warming to 60–70° C. with occasional shaking until a yellow precipitate is formed. After cooling and filtering, the clear light yellow filtrate is transferred to a suitable flask fitted with a fractionating column and refluxed for 10–15 min. in the column before the distillate is allowed to come over. The watery first fraction, 90–110° C., separates into two layers, of which the top one can be returned to the residue bottle. The fraction distilling between 128 and 132° C. is collected for re-use, but the purification may need to be repeated, if undue smell of sulphur compounds persists, as these are suspected of causing the iron colour to fade. Sulphur compounds are usually evident in the last high-boiling portion which should be returned to the residue bottle.

DISCUSSION

Although the original methods developed in the authors' laboratory gave satisfactory agreement between filtration and wet-ashing procedures, changes were necessary to provide a combined method whereby both iron and copper could be estimated upon the same sample. The amount of initial extraction acid has been increased to ensure complete solution of the metals, because on occasion particles of undissolved rust or verdigris may be present. Trials were carried out using up to 10 ml. of 6N-hydrochloric acid, or 8 ml. of concentrated hydrochloric acid, or 4 ml. of concentrated nitric acid, with and without reducing agents. Under the conditions of the method, neither concentrated hydrochloric nor nitric acid *alone* secures the complete extraction of iron, especially if large amounts are present. With more acid more iron can be extracted, but too much acid may interfere with the final estimation as thiocyanate(23). With too little acid, extraction of copper is also incomplete. Trials have shown that the use of less hyposulphite may give incomplete extraction of both metals, especially iron when large amounts are present; also that to ensure complete extraction the samples must be left long enough with the hyposulphite, preferably overnight in a warm incubator. If this reagent is stale it may give rise to low results, especially when large amounts of iron are present (see Table I, samples (*b*) and (*e*)).

In early trials with the combined method the filtrates were at times rather cloudy, and this cloudiness persisted in both acid and alkaline conditions. On shaking with the organic solvent a whitish turbidity gathered at the interface and the copper results were then rather low, while the iron colours developed a brownish tint and faded. With experience clearer filtrates were obtained, but recurrence of this trouble with odd samples, especially butter, led Mr F. Bishop to make a further study of filtration conditions. On comparing the aqueous volumes to be filtered from different samples, he noted that the volume from butters was about 13 ml., while from cream it was about 27 ml. Yet the filtration and washing of the cream samples was usually completed more readily and there was less trouble with cloudy filtrates. But the hydrochloric acid in the aqueous layer of the butter samples was appreciably stronger than for cream so further trials with various strengths of acid were made. For cream the acid could be increased to 8 ml. of 6N (or 5 ml. of A.R. acid which is about 10N). For butter the same amounts of acid may be used, but not more. The curd appears to separate more easily from the fat of butter samples if some water is added while they are being melted prior to adding the acid, using up to 11 ml. of water with 10N acid. If stronger acid is used for extraction of the metals, along with more

water, the increased volume enables the initial filtration to take place more readily so that less washing is needed. Quick filtration and washing are essential to getting satisfactory results. The protein precipitate is of a complex nature, containing tungstic acid, or perhaps phosphotungstic acid derived from soluble phosphates present in milk products. This complex precipitate appears to be easily hydrolysed if the degree of acidity is changed, e.g. when pure water is used for the final washing. On hydrolysis, some of the protein-tungstic acid complex passes into the filtrate which may become cloudy throughout, or cloudiness may develop when shaken with solvent to extract copper, giving low results. Trials have shown that the presence of tungstic acid in the filtrate is one of several factors which give rise to brownish tints and fading of the final iron colour. In this case the trouble seems to be due to a reaction between tungstic acid and peroxide, and to be accentuated also by bright sunlight. Tungstate must be added to give quick filtration and the amounts specified cannot be appreciably increased without risk of affecting the final estimation of iron. If low results are suspected to be due to filtration difficulties, the residues may be washed off the filter-papers into Kjeldahl flasks, and subjected to wet-ashing. Alternatively, an aliquot of the filtrate may be wet-ashed.

Cloudy filtrates were formerly suspected to be due to sulphur compounds passing into the filtrate, owing to insufficient time and temperature of heating after adding hyposulphite and prior to filtration. Because sulphite in the filtrate was suspected to cause fading of the iron colour, the addition of persulphate was formerly recommended, but this is not needed. With enough heating before filtration the use of peroxide suffices. Another turbidity evident only in the amyl acetate extract of one or two whey creams was similar to that produced by adding small amounts of tin salts to the usual standard solutions. Zinc may give a similar turbidity (19), but is less likely to be present than tin.

To develop the final copper colour, sodium diethyl-dithio-carbamate in solid form has been replaced by a 0·2% solution stabilized with ammonia, as advised by Clare, Cunningham & Perrin (21). The iron content of dairy produce is much lower than that of other biological samples, so that ammonium citrate need not be added to this reagent, especially if it has already been added to the filtrate and the solution then made strongly alkaline prior to the addition of carbamate.

For the combined filtration procedure, 5% hydrochloric acid as a washing agent has been found to give very slightly lower copper values. But if this acid be used for washing instead of nitric, iron may then be estimated in the ferrous condition by thioglycollic acid (13), or by α, α'-dipyridyl (17) or by *o*-phenanthroline (12) methods. In a series of comparisons of these reagents with thiocyanate, good agreement was obtained, but for several reasons we prefer the classical thiocyanate method, the mechanism of which has been clarified by several recent investigations (22, 23, 24, 25, 26).

Occasionally when the acid filtrate becomes slightly cloudy or coloured after standing, so that the aqueous solution is unsuitable for colorimetric estimation, extraction of the thiocyanate colour gives a clear liquid. Not only is the colour intensified by extraction into a small volume of alcohol, but also recent work (26) on the iron thiocyanate reaction suggests that whereas in aqueous solution the colour is due to the anion $Fe(CNS)_6'''$, extraction into hydroxylic or carboxylic solvents such as amyl alcohol or ether, favours the formation of a non-ionized complex $Fe_2(CNS)_6$, which is more intensely coloured and more stable. We have found that provided the amount of acid is not excessive, the extraction of the ferric thiocyanate into amyl alcohol leads to increased stability, so that after the removal

of the alcohol layer the full colour persists in it for many hours, long after the aqueous solution may have faded.

In dealing with two or three samples, the waiting time specified may at first prove tedious, but this is not the case when batches of two or three dozen are done at once, for the method as described usually gives quick filtration and clear filtrates, and the coloured extracts also separate and clear readily so that the final readings can be quickly completed.

The results of analyses carried out in the authors' laboratory during the past twelve years have shown that in addition to iron and copper present in cream or butter in a state of combination with the fat or the protein, minute particles of these metals or their insoluble compounds (e.g. oxides) may be present. Irregular distribution of such particles, and the difficulty of dissolving them in dilute acids, may at times give rise to discrepancies when replicate tests are done, especially if acids of different strength are being used, as in comparisons of the filtration and wet-ashing procedures (see iron figures in Table 1, samples (*b*) and (*e*); also Table 4, factory C). To even up discrepancies due to irregular distribution, the samples analysed should be as large as possible, and butter should not be melted before weighing. When the copper content is low, large samples are also desirable in order to provide a sufficiently intense colour in the final extract. For these reasons the 25 g. sample used in the combined method has definite advantages. A larger sample (50 g.) as used by Williams (8) and others, is in fact preferable to a smaller. Various minor difficulties may be encountered with either the filtration or the wet-ashing method, so that satisfactory results may not be obtained at the first attempt. Both methods have been applied to many samples over a period of years and after experience very reliable results can be obtained, especially with the filtration method if due care is taken about the points emphasized.

RESULTS

Comparisons have been made between analyses done by the combined filtration method and by the improved wet-ashing procedure. In Table 1 are given, as parts of copper and iron per million of sample, results obtained from a series of cream samples taken at different stages during the butter-making process. Butter from a certain factory had been found to contain a slightly higher iron content (up to 1·5 p.p.m.) than was usual for good creamery butter, and in order to investigate this, the series of cream samples was analysed.

In general, the values given in Table 1 for both copper and iron by the two methods agree satisfactorily. The higher iron values for samples (*b*) and (*e*) by the wet-ashing procedure were probably due to the fact that particles of rust or the like were present in the watery fluid. Complete solution of these particles was not effected because the acidic conditions of the filtration method were on that occasion insufficient (probably due to the hyposulphite solution being stale and weak). The results showed that the copper content of the raw cream was quite low, but the first gallon over the plant picked up a small amount. Corrosion of brass about the tap was probably responsible for the higher copper values of sample (*e*), as later figures were quite satisfactory. For the iron figures, the raw cream had a satisfactory iron content, but the first gallon over the plant showed a considerable increase and the first gallon from the cold vat also had a high value. Although a considerable amount of the copper and iron went away in the buttermilk, the iron content of the butter was a shade high but the copper content was satisfactory. The high iron values for samples (*b*) and (*e*) seemed sufficient to account for the iron content of this particular brand of

butter, and provided ample evidence to support the recommendation for liberal rinsing of the plant with water before the cream began to flow, and also for discarding a gallon or more of the watery liquid from the cold tap, before the cream was pumped to the churn.

Table 1. *Comparison of methods*

	Filtration method		Wet-ashing method	
Nature of sample	Copper (p.p.m.)	Iron (p.p.m.)	Copper (p.p.m.)	Iron (p.p.m.)
(*a*) Raw cream from receiving vat	0·13	0·79	0·11	0·84
	0·13	0·75	0·13	0·88
	0·14	0·83	0·09	0·82
(*b*) First pasteurized cream flowing to cold vat (watery)	0·22	2·31	0·26	2·52
	0·20	2·35	0·29	2·62
	0·22	2·35	—	2·62
(*c*) Later cream (after 50–60 gal.) to cold vat	0·14	0·91	0·17	1·04
	0·15	0·93	0·16	0·92
	0·15	0·93	0·17	0·94
(*d*) Later cream (after 150–200 gal.) to cold vat	0·14	0·47	0·15	0·46
	0·14	0·37	0·14	0·64
	0·14	0·43	—	—
(*e*) From cold vat tap, before pasteurized cream stirred (watery)	0·31	2·70	0·30	3·52
	0·30	2·70	0·28	3·48
	0·30	2·38	0·32	3·16
(*f*) First outflow to churn	0·15	1·25	0·14	1·20
	0·14	1·17	0·14	1·34
	0·14	1·33	0·13	1·30
(*g*) Outflow to churn after 100 gal.	0·14	1·09	0·13	1·06
	0·14	1·15	0·11	0·98
	0·14	1·13	0·13	1·10
(*h*) Buttermilk half-way through run-off	0·22	1·33	0·19	1·30
	0·22	1·35	0·21	1·32
	0·23	1·29	0·20	—
(*i*) Butter	0·16	1·06	—	—
	0·16	1·06	—	—
	0·14	0·96	—	—
	0·14	1·00	—	—

As a further check on the reliability of the combined filtration method, known amounts of copper and iron were added to samples of butter, etc., and recoveries of the added metal were made by this method with very satisfactory results. These are shown in Table 2, where the figures (except those for cream) are averages of duplicates. Less satisfactory recovery was obtained when less acid was used with some of these samples. The variations in the iron results of the cream replicates fall within 0·3 units of the galvanometer scale, which is very satisfactory.

To investigate the occurrence of excessive metallic contamination in whey butter, whey cream supplies from individual cheese factories have been examined, in addition to samples, similar to those in Table 1, taken at different stages throughout the plant. In Table 3 are copper and iron figures given by whey creams from twelve different cheese factories. Replicate portions of these samples were examined by both the filtration and the wet-ashing methods, the combined procedure being used in each case so as to give both the copper and the iron figure from each 25 g. portion. The results by the two methods show quite satisfactory agreement. The slight variations in the iron figures may be partly due to irregular distribution of rust particles.

The following figures were obtained from samples of whey cream sent by ten suppliers

to a factory, from which whey butter was found on several occasions to contain about 0·3 p.p.m. copper and more than 6 p.p.m. iron:

Copper (p.p.m.)	0·16	0·18	0·20	0·27	0·22	0·16	0·24	0·27	0·23	0·33
Iron (p.p.m.)	4·16	7·55	9·41	2·47	2·22	4·08	16·60	4·11	3·60	7·65

Table 2. *Recovery trials, filtration method*

	Copper (p.p.m.)			Iron (p.p.m.)		
Sample	Added	Found	Expected	Added	Found	Expected
Butter (1)	Nil	0·19	—	Nil	1·80	—
	0·50	0·71	0·69	0·20	1·97	2·00
	0·40	0·57	0·59	0·50	2·30	2·30
	0·30	0·45	0·49	0·80	2·57	2·60
	0·15	0·33	0·34	1·00	2·90	2·80
	0·10	0·25	0·29	1·50	3·40	3·30
Butter (2)	Nil	0·56	—	Nil	3·13	—
	0·1	0·64	0·66	0·30	3·15	3·13
	0·2	0·74	0·76	—	—	—
	0·3	0·83	0·86	0·70	3·80	3·83
	0·4	0·96	0·96	—	—	—
	0·5	1·02	1·06	1·00	4·12	4·13
	0·8	1·28	1·36	2·00	5·17	5·13
	1·0	1·50	1·56	1·50	4·58	4·65
	2·0	2·29	2·56	3·00	6·22	6·13
Cream	Nil	0·12	—	—	4·64	—
	0·10	0·21	0·22	—	4·60	—
	0·20	0·29	0·32	—	4·60	—
	0·30	0·40	0·42	—	4·53	—
	0·40	0·49	0·52	—	4·53	—
	0·50	0·56	0·62	—	4·68	—
Buttermilk	Nil	0·23	—	Nil	1·47	—
	0·3	0·50	0·53	1·0	2·41	2·47
	0·5	0·69	0·73	0·5	1·95	1·97

Table 3. *Whey creams by two methods*

	Copper (p.p.m.)		Iron (p.p.m.)	
Factory	Filtration	Wet-ashing	Filtration	Wet-ashing
A	0·11, 0·16, 0·10	0·16	1·44, 1·56, 1·38	1·62
B	0·21, 0·22	0·20	8·00, 7·80	6·50
C	0·24, 0·22	0·26	4·20, 5·50	5·00
D	0·15, 0·15	0·20	5·50, 5·95	5·95
E	0·13, 0·12	0·15	5·70, 6·05	5·55
F	0·26, 0·20	0·28	6·50, 6·66	6·25
G	0·12, 0·13	0·15	8·00, 8·00	8·20
H	0·12, 0·12, 0·12	0·15	7·60, 7·60, 7·80	6·75
I	0·26, 0·28	0·29	2·85, 2·80	3·10
J	0·08, 0·09	0·11	2·70, 2·70	3·15
K	0·13, 0·13, 0·12	0·14	4·50, 4·20, 4·50	5·20
L	0·08, 0·08, 0·08	0·09	2·80, 3·18, 3·24	2·90

In contrast to these are the results given by whey cream from suppliers to another factory which was making whey butter consistently low in both metals. Five whey butter samples during a period of about 2 years gave copper figures of 0·22–0·26 p.p.m., and iron figures of 1·3–2·1 p.p.m. The figures for the whey cream supplies were as follows:

Copper (p.p.m.)	0·17	0·16	0·19	0·34	0·16	0·13	0·14	0·13
Iron (p.p.m.)	1·21	2·85	2·76	2·62	1·98	3·20	3·10	2·26

For reasons already mentioned the results obtained from samples with high iron content may not be quite so accurate. Nevertheless, very satisfactory agreement can be obtained as is shown in Table 4, where the values given for factory A and B in each case represent

two sets of duplicate determinations upon whey creams by the filtration method. The copper figures for factory C are very uniform, but the iron figures vary considerably, probably due to the presence of particles of rust.

Table 4. *Whey creams with high iron content*

Factory	Copper (p.p.m.)		Average	Iron (p.p.m.)		Average
A	0·21	0·22	0·23	31·8	31·4	31·5
	0·23	0·25		32·6	30·2	
B	0·19	0·19	0·20	6·70	6·60	6·68
	0·21	0·19		6·90	6·50	
C	0·43	0·48	0·45	10·3	15·5	—
	0·45	0·46		10·0	180·0	
				9·6		

On one occasion the filtration method was applied to a sample of white whey from the cheese presses. As is usual for such a sample it was high in salt, with the result that the portion for iron faded quickly to an orange-brown tint on the addition of thiocyanate. The true colour was restored by the addition of hydrogen peroxide. On repeating the analysis with less initial hydrochloric acid the same effect was observed. The colour was again restored with peroxide, but the results were rather lower, 4·2 and 4·6 as compared with 5·2 and 6·8 p.p.m. previously.

Buttermilk provides a very suitable material for use in comparing the reliability of different analytical procedures because it has a low fat content (about 1%). The analytical operations can therefore be carried out without the hazards associated with the disposal or removal of the fat in one way or another. In Table 5 are given the results obtained by

Table 5. *Replicate analyses of butter-milk samples*

Factory	Group letter	Copper (p.p.m.)		Iron (p.p.m.)	
		Filtration	Wet-ashing	Filtration	Wet-ashing
1	A	0·24	0·23	1·21	1·23
		0·22		1·28	
		0·23		1·24	
		0·24		1·18	
2 (from whey butter)	A	0·36	0·32	2·60	2·56
		0·34		2·61	
		0·35		2·62	
		0·34		2·79	
3	B	0·26	0·26	1·22	1·20
		0·26		1·22	
	C	0·25	0·25	1·20	1·23
4	B	0·49	0·47	1·35	1·40
		0·46		1·38	
	C	0·52	0·53	1·46	1·53
5	B	0·17	0·16	1·57	1·71
		0·16		1·54	
	C	0·14	0·14	1·65	1·65
6	B	0·16	0·16	1·87	—
		0·15		1·80	
	C	0·15	0·16	2·00	2·13
7 (from whey butter)	B	0·20	0·18	1·73	1·85
		0·19		1·74	
	C	0·19	0·20	1·70	1·55

varying the analytical methods applied to buttermilk samples from different factories which were dealt with in three groups, A, B and C. In the case of group A, samples 1 and 2,

five portions of each were analysed, the filtration method being applied to four; to the fifth the wet-ashing method was applied after pipetting the sample into a small Kjeldahl flask, from which the completed digest was later transferred to a tube to be dealt with as already described. With samples 3–7 two portions of each, group B, were first analysed by the filtration method and at the same time a third was subjected to wet-ashing in the same way as described for samples 1 and 2. Then two further portions (group C) from each of these five samples were analysed, one of each pair by the filtration method; the other of each pair was pipetted direct into 8 × 1 in. Pyrex test-tubes for wet-ashing. The difficulties associated with transfer of the final digest to other tubes were avoided by adding the reagents required and making up 50 ml. in these Pyrex tubes, prior to removing 10 ml. for iron, and completing as usual. The results in Table 5 show very good agreement between analyses replicated in this way, and demonstrate that the residue from wet-ashing in Kjeldahl flasks can be transferred to other tubes without significant loss.

Table 6. *Butter and buttermilk results*

Factory		pH	Copper (p.p.m.)	Iron (p.p.m.)
A1	Butter	7·2	0·10	0·59
	Buttermilk		0·41	0·89
A2	Butter	7·3	0·19	0·54
	Buttermilk		0·68	0·78
A3	Butter	7·2	0·14	0·65
	Buttermilk		0·61	1·10
A4	Butter	7·3	0·16	0·42
	Buttermilk		0·52	0·70
B1	Butter	7·0	0·10	0·61
	Buttermilk		0·18	1·14
C1	Butter	7·2	0·19	0·54
	Buttermilk		0·30	0·29
C2	Butter	7·2	0·17	0·44
	Buttermilk		0·26	0·32
D1	Whey butter	7·5	0·25	2·60
	Buttermilk		0·20	2·08
E1	Whey butter	7·9	0·43	3·38
	Buttermilk		0·47	3·04
F1	Whey butter	8·2	0·43	3·42
	Buttermilk		0·37	1·22

In Table 6 are given the average figures from duplicate tests by the filtration method upon a series of samples of butter and their respective buttermilks. The figures show that the buttermilk usually collects a larger proportion of the total copper than of the iron. This effect is not so pronounced in Table 1, samples (*h*) and (*i*). In each case values for pH of the butter are given, as that appears to be one of several factors affecting the distribution of the metals between butter and buttermilk. In the case of whey butter, insoluble particles of rust or verdigris are probably gathered into the butter so that the proportion of metal which goes away in the buttermilk is not as high as with creamery butter.

In Table 7 are summarized the separate copper and iron results of duplicate analyses upon about 275 samples of creamery butter and nearly 160 samples of whey butter which were examined by the combined filtration procedure during a period of about 2 years. In the classification column are the terms used in reporting the results obtained from creamery butter; for whey butter, rather higher limits are allowed for the same terms.

Table 7. *Summarized results*

	Creamery butter			Whey butter	
Copper present (p.p.m.)	Creamery classification	Number	Percentage	Number	Percentage
0·0 –0·10	Good	64	23	3	2
0·11–0·15	Satisfactory	128	46	8	5
0·16–0·20	Doubtful	50	18	24	15
0·21–0·30	Unsatisfactory	26	9	68	43
0·31–	Excessive	10	4	54	34
Iron present (p.p.m.)					
0·0 –0·50	Very good	81	29	—	—
0·51–1·00	Good	139	50	—	—
1·01–1·50	Satisfactory	39	14	11	7
1·51–2·00	Doubtful	11	4	23	14·5
2·01–3·00	Unsatisfactory	3	1·1	38	24
3·01–5·00	Excessive	2	0·7	68	43
5·01–	Excessive	—	—	18	11·5

Since either copper or iron may stimulate the deterioration of butter, the analytical results may be grouped to indicate the proportion of samples which are satisfactory or otherwise with respect to *both* metals. On this basis, about 63% of the creamery butter samples may be classed as very satisfactory with a low content of both metals, while about 14% have either high copper or high iron. When similarly grouped, only about 14% of the whey butter samples are really satisfactory, while fully 40% are exceedingly high with respect to at least one of the metals.

Further notes

The wet-ashing method has been applied to estimate the copper content of cheese for which, of course, the filtration method is unsuitable. With normal cheese, 5 g. samples were digested with 2 or 3 ml. of sulphuric acid and nitric acid as required, and results of 2–3 p.p.m. of copper were obtained. These samples were obtained from a factory using a plate-type regenerative pasteurizer, of which the copper plates had just been retinned. Previous samples of cheese from this factory were submitted for analysis because loss of tin from the plates was thought to be the cause of increasing the copper content of the milk to such an extent that the growth of the starter bacteria in the cheese vats was checked. After preliminary trials with larger amounts, digestion of 1 g. samples from different vats yielded values ranging from 22 to 75 p.p.m. of copper. These high values were obtained from cheese made in the vat containing most of the first milk run through the pasteurizer in the morning. On several days this vat with the high copper content worked more slowly than the rest and the resulting cheese was inferior in quality to the remainder of the factory output.

Since this paper was prepared, some trials of the filtration method upon milk have been made by Mr J. E. Allan who has recently used kinetic methods to investigate the effect of copper and iron upon the oxidation of ascorbic acid in butter serum (28). To obtain satisfactory filtrates more tungstate is needed and also more citrate for copper. The principal difficulty is due to the fact that the protein in milk yields a precipitate which is very different in character from that given by buttermilk from cream which has been pasteurized at very high temperatures. Curd from the latter is granular and easily washed, as distinct from the compact curd which gathers in the filter-paper from milk. The latter is very

difficult to wash, with the result that recovery of added metal is only about 70–90%. A modification of the wet-ashing method may be preferable.

With regard to milk powder, preliminary comparisons by Mr F. Bishop have shown that the filtration method can be easily applied to 1 g. samples and is capable of giving results which agree quite well with those obtained by wet-ashing. For the prolonged wet digestion which may be needed, Pyrex glass is preferable as traces of iron may be extracted from certain other types of glass.

SUMMARY

1. Improvements are described in the methods for the extraction and colorimetric estimation of metals in dairy produce, using the carbamate reagent for copper and thiocyanate for iron.

2. A combined filtration procedure has been developed to enable both metals to be determined on the same sample, and with slight modifications this procedure has been applied to the analysis of samples of butter, cream, buttermilk and whey.

3. The wet-ashing technique has also been improved to enable it to be used for the estimation of both metals on the same digest.

4. Comparisons show that the combined filtration method is capable of giving results which agree very well with those yielded by wet-ashing.

5. The results of many analyses show that the majority of creamery butter samples have a satisfactorily low content of both copper and iron.

6. Many whey butter samples have a rather high metallic content.

7. Notes are given on the results obtained when the methods described are applied to other dairy produce.

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438. THE VISCOSITY TEMPERATURE COEFFICIENT OF HOMOGENIZED MILK

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(With 13 Figures)

(1) INTRODUCTION

Some preliminary experiments were carried out to measure the viscosity-temperature coefficient of milk using an Ostwald viscometer. To obtain accuracy, it is necessary to have a relatively large efflux time. If a viscometer is chosen to meet this requirement, creaming occurs during the course of a single run, thus leading to an error in the value of the viscosity. It would appear that this has been overlooked in past work, and as it is desirable to have an accurate value for the viscosity-temperature coefficient, it was decided to redetermine this coefficient. No obvious method is available to overcome this difficulty; it was decided to carry out experiments on homogenized milk where this effect would be minimized. It was realized that this would not give the same viscosity as that of raw milk, but it was felt that the temperature viscosity coefficient might be similar.

The method of measurement was chosen so that an overall accuracy of 1% could be obtained, which was believed to be better than in the past.

(2) METHOD

The method was essentially a capillary tube one, in which an external pressure was applied to force the liquid through a capillary tube. The time (t) for a given volume(V) of liquid to pass through the capillary was recorded, and the viscosity was calculated from Poiseuille's equation

$$\eta = \frac{\pi R^4 pt}{8Vl} - \frac{m\rho V}{8\pi lt}, \tag{1}$$

where R is the radius of the capillary tube, p the effective pressure, l the length of the capillary tube, ρ the density of milk and m a constant. There is some doubt as to the correct value of m for simple liquids, and there has been no measurement of m for the complex biological fluid milk. It was decided to minimize the kinetic energy correction, i.e. the term involving m in equation (1) by making the time of flow large. Thus preliminary calculation showed that the time for 10 c.c. of water at 20° C. to flow through the tube was of the order of 3000 sec., with an applied pressure of 15 cm. of mercury, a capillary tube of length 20 cm. and radius 0·01 cm. The kinetic energy correction assuming $m=1$, was of the order of 0·00001, which amounts to a correction of one part in a thousand to the viscosity. The corresponding Reynolds number was of the order of a hundred; thus laminar flow was ensured.

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(3) DESCRIPTION OF THE APPARATUS

The apparatus is shown diagrammatically in Fig. 1. The bulbs A and B contain milk and were so constructed that when the milk was at the level G_1 in A it was at G_4 in B, and conversely when at G_2 in A it was at G_3 in B. The source of applied pressure was high-pressure nitrogen in a cylinder S connected through an adjustable reducing valve V to the reservoir R, and thence to a four-way tap T by means of rubber pressure tubing. The pressure in the reservoir R was controlled by means of a mercury bubbler D. In the rubber tubing connecting the reservoir R with the four-way tap T, there was inserted a tightly packed plug of cotton-wool W, which reduced considerably the pressure fluctuations produced by the bubbles of escaping nitrogen in the mercury bubbler.

The viscometer bulbs, A and B, had side arms, and to these arms there was connected a U-tube of diameter 1·0 cm. containing mercury which acted as a manometer. The applied pressure was read from this manometer by using a cathetometer. There were two constrictions in each bulb and at each constriction there was a fiduciary mark (G_1, etc.)

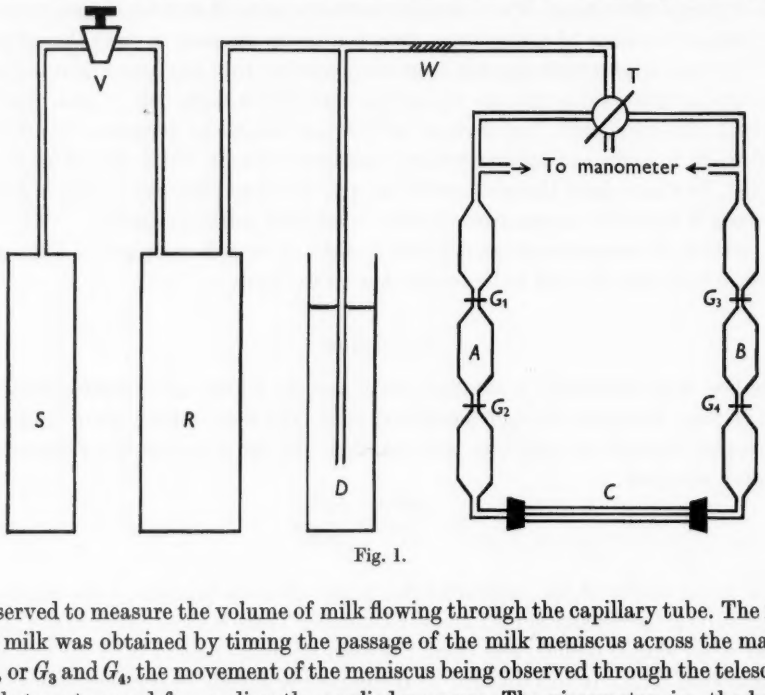

Fig. 1.

which served to measure the volume of milk flowing through the capillary tube. The rate of flow of milk was obtained by timing the passage of the milk meniscus across the marks G_1 and G_2, or G_3 and G_4, the movement of the meniscus being observed through the telescope of the cathetometer used for reading the applied pressure. The viscometer, i.e. the bulbs A and B and the connecting capillary tube C, was attached to a rigid frame and immersed in a water-bath. The water-bath could be kept at any temperature from 20° to 80° C. and could be maintained at that temperature to within $\frac{1}{50}$° C.

(4*a*) MEASUREMENT OF THE RADIUS OF THE CAPILLARY TUBE

Twelve 30 cm. lengths of 'Veridia' precision glass tubing were examined. Each tube was nearly filled with mercury, and the length of the thread measured. The mercury thread was weighed, and hence a mean radius could be calculated. From the twelve lengths

three were chosen whose radii agreed amongst themselves to within two parts in a thousand. A second and more careful determination of the mean radius on each of the three lengths was made as follows. First the tube was nearly filled with clean dry mercury. The length of this thread was measured using a cathetometer and the temperature at which the length measurement was made was recorded. The mercury was then carefully transferred to a weighed beaker in a desiccator. A second thread was now drawn into the tube, its length measured and the mercury added to that in the weighed beaker. This was repeated a third time giving in all about 0·35 g. of mercury. The weighings were made on an analytical balance reading to $\frac{1}{10}$ of a milligram. The whole procedure was repeated five times for each tube. The mean radius was calculated from the formula

$$r^2 = W/\pi l\rho, \tag{2}$$

where r is the radius of the tube, W is the mass of mercury, l is the total length of the thread of mercury and ρ the density of mercury at the appropriate temperature. The error in r^2 has three contributory factors, namely (a) the error in W, (b) the error in l and (c) the error in ρ. From (2) we have

$$\frac{\delta r^2}{\delta W} = \frac{1}{\pi l\rho}, \tag{3}$$

$$\frac{\delta r^2}{\delta l} = -\frac{W}{\pi l^2\rho}, \tag{4}$$

$$\frac{\delta r^2}{\delta \rho} = -\frac{W}{\pi l\rho^2}; \tag{5}$$

whence

$$\sigma(r^2) = \frac{W^2}{\pi^2 l^2 \rho^2}\left[\frac{\sigma(W)}{W^2} + \frac{\sigma(l)}{l^2} + \frac{\sigma(\rho)}{\rho^2}\right], \tag{6}$$

where $\sigma(r)$ is the variance of r, etc. The standard error of W was 0·0002 g. The standard error of l was 0·009 cm. arising from an error at each end of the tube, and the standard error of ρ was 0·005 equivalent to an error of temperature of 2° C. The variances are the squares of the standard errors, taking then $l = 90$ cm., $W = 0{\cdot}35$ g. and $\rho = 13{\cdot}55$ we obtain $(r^2) = 3{\cdot}6 \times 10^{-15}$, or S.E.M. $(r^2) = 6 \times 10^{-8}$ cm.^{2} The experimental value of the S.E.M. (r^2) determined from the five replicate values of r^2 was 9×10^{-8} cm.^{2} showing close agreement with the calculated value above. Thus the percentage error of r^2 is approximately 0·1%, and therefore the percentage error in r^4 will be of the order of 0·2%.

(4*b*) MEASUREMENT OF THE LENGTH OF THE CAPILLARY TUBE

From one 30 cm. length of tubing a 10 cm. length was cut and its ends ground flat and square. The tubes were ground on dry emery paper whilst nitrogen was blown through the capillary tube. This technique prevents small fragments of glass from entering the capillary tube and adhering to the inside wall. The length was then measured at room temperature using the cathetometer. Twenty readings of the length gave a mean of 10·204 cm. S.E.M. 0·001 cm. giving an accuracy of better than 1 part in 10,000.

(4*c*) THE MEASUREMENT OF THE APPLIED PRESSURE

The manometer was constructed of 1·0 cm. diameter tubing. When there was no applied pressure, there could not be detected any difference in level of the mercury surface. If then there were any unbalanced surface tension forces, giving rise to differences in mercury

level, these were masked by the slight errors in levelling the cathetometer. The menisci were shaded by black paper bands round each tube to prevent reflexion from the mercury surface, thus giving a clear outline. The levels of the mercury were read by a cathetometer. The cotton-wool plug reduced the pressure fluctuations to the order of 0·002 cm. of mercury. The manometer was not immersed in the thermostat, but outside, and the room temperature was frequently recorded during the course of an experiment..

(4*d*) THE MEASUREMENT OF TEMPERATURE

The temperature of the water-bath was read on a N.P.L. calibrated mercury-in-glass thermometer reading to $\frac{1}{5}$° C. The position of the mercury thread in the thermometer was read using the same cathetometer as was used for the pressure measurements; thus $\frac{1}{100}$° C. could be recorded, although the absolute accuracy of the calibration was not better than $\frac{1}{20}$° C.

The room temperature was read on an ordinary 0°–100° C. laboratory thermometer reading to degrees.

(4*e*) THE MEASUREMENT OF TIME

The time for the volume of milk between the fiduciary marks to flow through the capillary tube was recorded on a stop-watch with a fifth movement. The watch was checked against a good clock which was checked against the B.B.C. time signal and an appropriate correction factor determined.

(4*f*) THE MEASUREMENT OF THE VOLUME BETWEEN THE FIDUCIARY MARKS

The bulb was filled with clean dry mercury until the level corresponded with the mark G_1 as viewed through a telescope. The mercury was run out into a weighed beaker till the mercury level coincided with G_2. The mercury was weighed, and using the density of mercury at the room temperature, the volume between the fiduciary marks was calculated. Ten replicate readings were taken on each bulb giving a mean volume 9·973 c.c. ± S.E.M. 0·003 c.c. and 10·093 c.c. ± 0·002 c.c. respectively.

(5*a*) THE CORRECTION DUE TO UNIFORMITY OF BORE

Bond (1) has shown that variation of radius along the length of the capillary tube introduces a correction to the factor $\int \frac{dl}{r^4}$ of the form $[1/q]^2[q^2]$ where $[q^2]$ is the mean square length of a short thread of mercury measured at successive positions along the tube, and $[1/q]$ is the mean reciprocal length of this thread. In this work a thread of mercury 1 cm. long was introduced into the 30 cm. length of capillary tubing, and its length read at successive positions in the tube giving thirty readings in all. Those readings falling within the 10 cm. length were used to calculate Bond's correction. This amounted to 1·0006 and for a 0·5 cm. thread, 1·0007. As both correction factors are less than 1 part in 1000 they were neglected.

(5*b*) THE CORRECTION DUE TO HYDROSTATIC HEAD

The applied pressure is not the pressure to use in calculating the viscosity from equation (1) because of the varying hydrostatic head of the milk. This correction has been carefully

investigated (2), and it has been shown that for viscometer bulbs of the shape used in this work equation (1) can be written

$$\eta C = \frac{\pi R^4 p_0 t}{8vl}, \tag{7}$$

where p_0 is now the applied pressure and C is a constant depending on the dimensions of the viscometer and p_0. The constant has been calculated for values of $p_0 = 25$ cm. of mercury, and the correction is less than 1 part in 1000.

The pressure drop in the bulbs A and B can be calculated from the radii and the lengths of the bulbs for a mean value of the viscosity of milk and is negligible. Thus the pressure drop across the capillary is represented to within 1 part in 1000 by the applied pressure.

(5*c*) CORRECTION DUE TO THE KINETIC ENERGY

It was decided to use the value $m = 1$ for the constant appearing in the kinetic energy correction term of equation (1). This correction varies in magnitude with the time of flow through the capillary tube. At the highest temperatures it amounts to approximately a 1% correction to the viscosity, whilst at 20° C. to a 0·1% correction.

(6) EXPERIMENTAL TECHNIQUE

Homogenized milk was supplied by the Express Dairy Co. Ltd., and immediately on arrival it was placed in a cold store at 0° C. The capillary tube and bulbs were cleaned in *aqua regia*, washed with water, rinsed with alcohol, and dried with ether before each new sample was placed in the viscometer. The milk was taken from the cold store and the viscometer was filled. Before placing the viscometer in the water-bath, the pressure was adjusted to a suitable value and the supply of high-pressure nitrogen was just sufficient to keep the bubbler bubbling slowly and steadily. The viscometer was placed in the water-bath and the manometer was connected to the bulbs A and B which were connected to the tap T. After a fixed time, usually 10 min., the run was commenced. The manometer was read twice during the run and the temperature of the bath also read twice. In a usual case, three further runs were made on the same sample of milk. This routine was followed at all temperatures.

The composition of the bulk supply was determined and the value of the density calculated for any temperature using the tables given by J. T. Bowen (3).

(7) DISCUSSION OF RESULTS

A series of replicate tests on a single bulk sample were made. Two successive readings being taken on each sample. The results are shown in Table 1.

Table 1

Viscosity (poise × 10^3)	Temperature (° C.)	Date	Time
7·96	59·5	19. ix. 47	2.30 p.m.
8·05	59·5		
7·94	59·9	19. ix. 47	3.40 p.m.
8·02	59·9		
8·38	59·5	19. ix. 47	5.0 p.m.
8·34	59·5		
8·59	59·9	20. ix. 47	9.35 a.m.
8·67	59·9		
8·51	59·9	20. ix. 47	11.10 a.m.
8·62	59·9		
9·01	59·9	20. ix. 47	12.0
9·11	59·9		

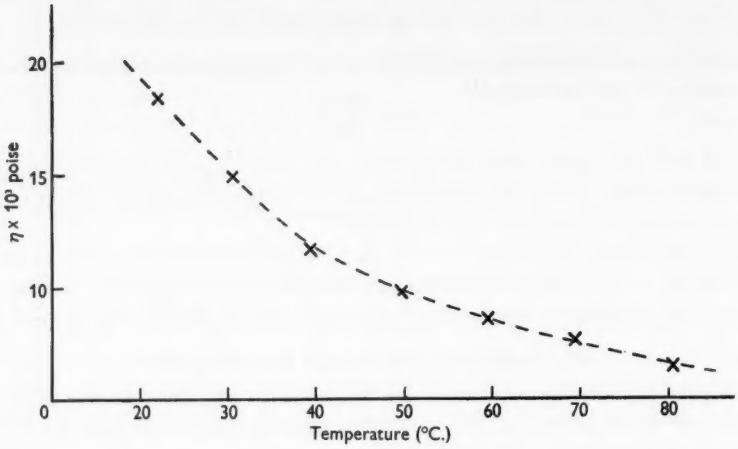

Fig. 2. Sample A.

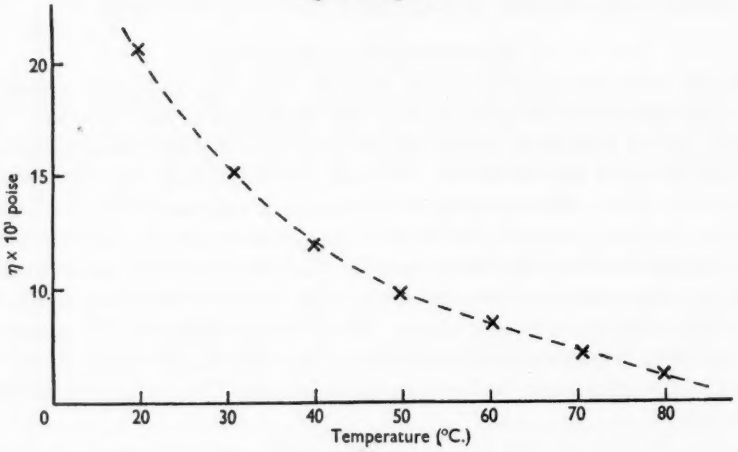

Fig. 3. Sample B.

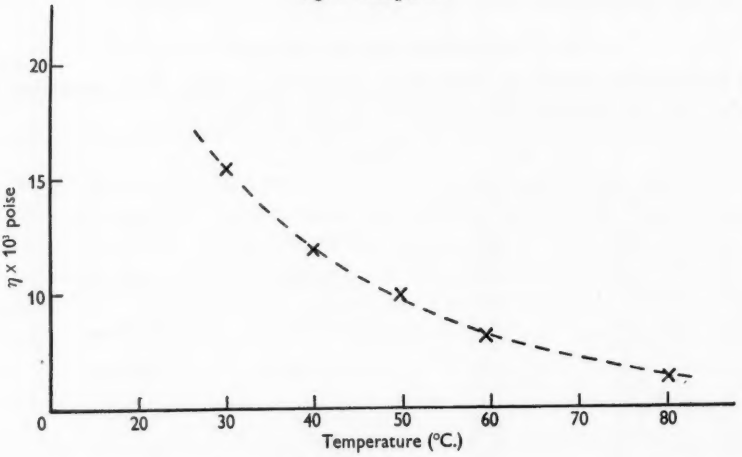

Fig. 4. Sample C.

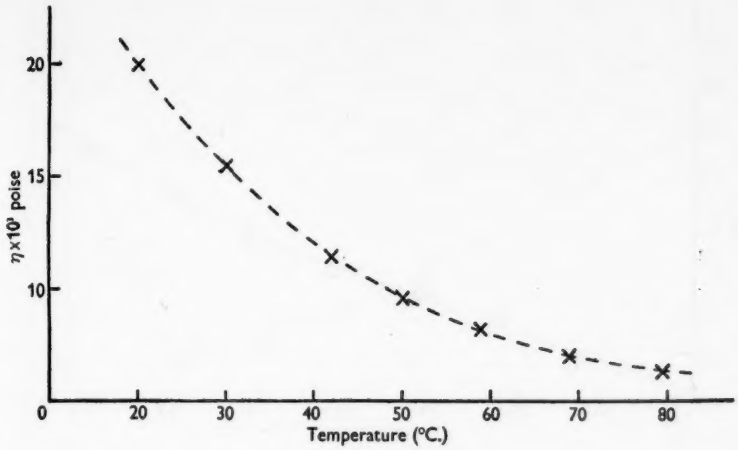

Fig. 5. Sample D.

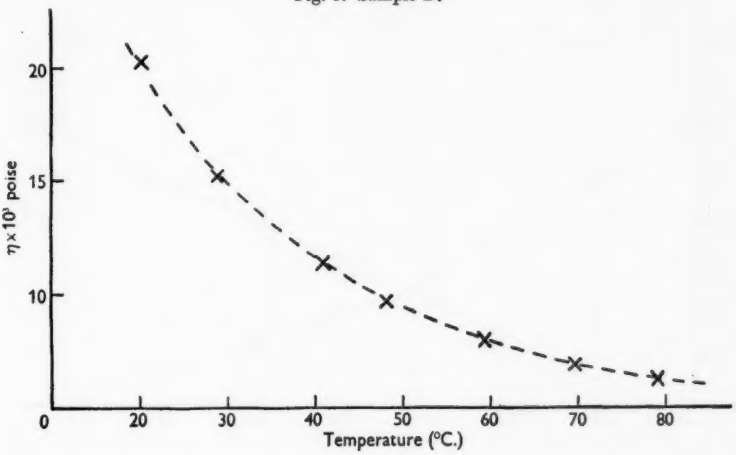

Fig. 6. Sample E.

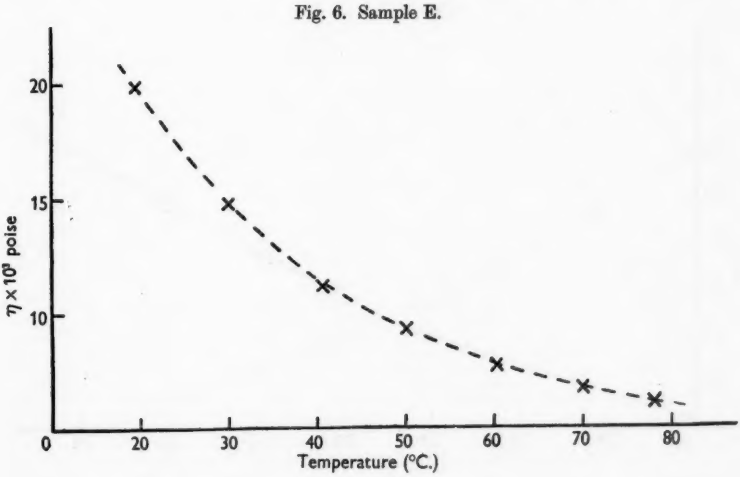

Fig. 7. Sample F.

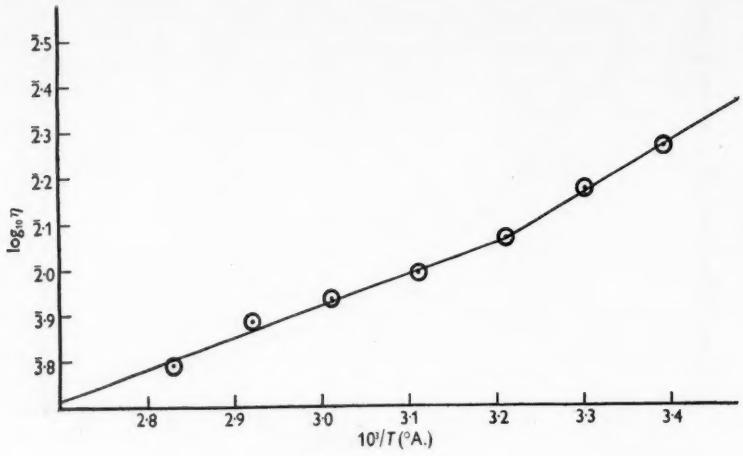

Fig. 8. Sample A.

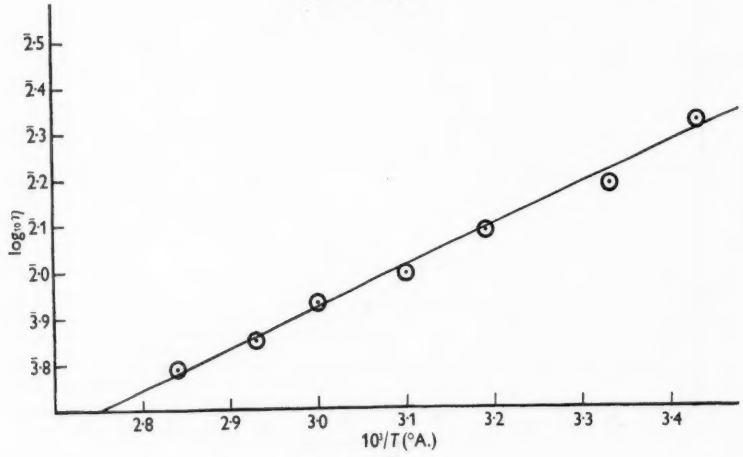

Fig. 9. Sample B.

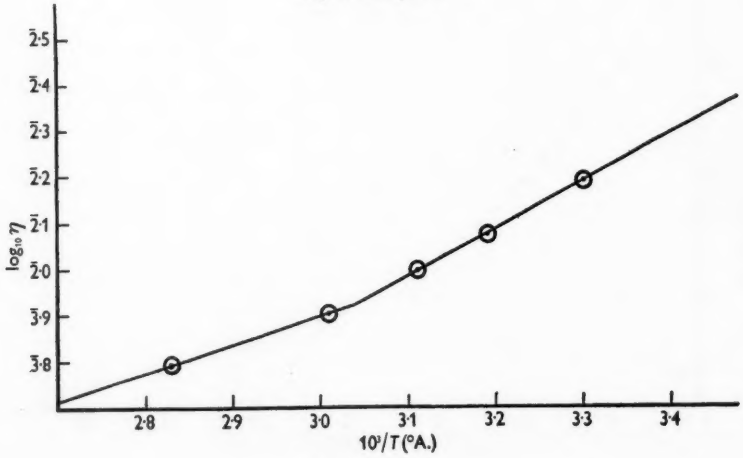

Fig. 10. Sample C.

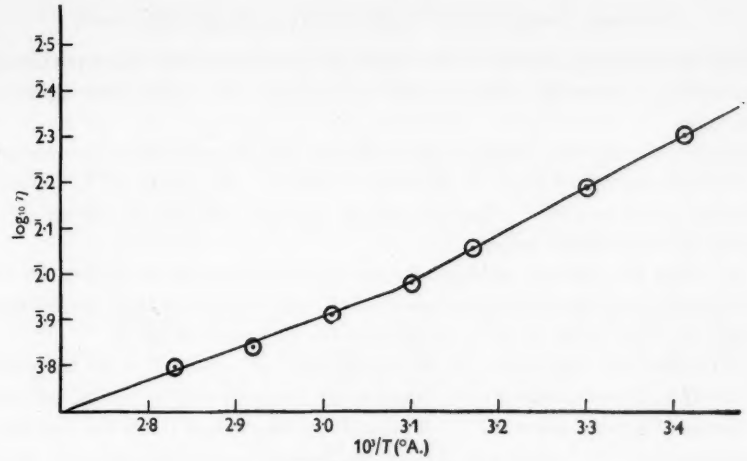

Fig. 11. Sample D.

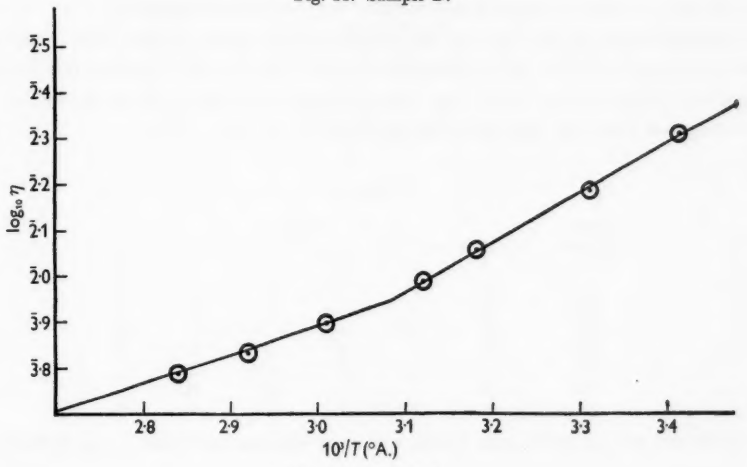

Fig. 12. Sample E.

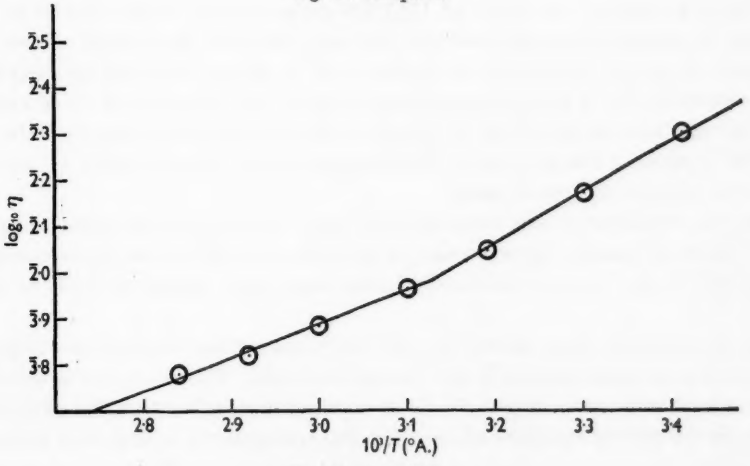

Fig. 13. Sample F.

From the table it is apparent that the viscosity rises steadily, and the repeat reading on the same sample is generally higher at this temperature. The latter phenomenon will be discussed later.

To determine the viscosity temperature coefficient the following procedure was adopted. The first sample was tested at 20° C., the second at 40° C., etc., up to 80° C. Then samples were tested at 70, 50 and 30° C., thus any drift in viscosity with time of storage was spread over the whole temperature range.

Figs. 2–7 show the viscosity calculated from the first run plotted against the temperature for six bulk samples. The curves are smooth, and it appears that the viscosity falls from about $2{\cdot}0 \times 10^{-2}$ poise at 20° C. to about $6{\cdot}0 \times 10^{-3}$ poise at 80° C.

Figs. 8–13 show the logarithm of η plotted against $1/T$, where T is the temperature in degrees absolute. These curves are not linear as are those for simple liquids, and appear to show a change of slope at about 40° C. A possible explanation of this is the fact that as the milk was stored at 0° C. before test, the fat was probably solid and this solid fat took an appreciable time to melt even at temperatures above its melting-point.

Four successive runs were made on each milk at each temperature. From inspection of the data it is apparent that at temperatures below 60° C. most samples fall in viscosity with shearing, whilst above 60° C. the viscosity tends to rise. This is shown for the six complete tests on different bulk samples in Table 2.

Table 2

Temp. of test (° C.)	Sample 1	2	3	4	5	6
20	F	F	–	F	–	F
30	F	–	–	F	F	F
40	F	–	–	–	F	F
50	F	–	F	F	F	F
60	–	–	F	F	F	–
70	R	–	–	R	R	–
80	R	R	R	R	R	R

F = fall in viscosity with successive runs; R = rise in viscosity with successive runs; – = no systematic trend.

The effect is small (of the order of 1%), but quite definite. It was decided to investigate this. A sample of homogenized milk was tested at 60° C. and twelve successive runs were made. A second sample was also tested at 60° C., tested twice and then left to stand in the viscometer for a time corresponding to eight runs, then tested twice again. The successive runs show an initial fall in viscosity then a rise, while the sample tested after a rest at 60° C. shows a rise in viscosity. This experiment was repeated at 80° C., and in both cases a rise of viscosity was obtained.

These two experiments were repeated with super-centrifuged skim milk, and at 60° C. all the values of viscosity agreed amongst themselves to within the experimental error, while at 80° C. the viscosity increased whether repeatedly sheared or if left to stand for a time.

It can be seen from these results that the rise in viscosity at temperatures above 60° C. occurs with both super skim milk and homogenized milk. This rise is also apparent if the sample is kept sufficiently long at 60° C. It is probable therefore that this effect is due to changes in the protein structure of the milk. The homogenized milk showed an initial fall in viscosity with successive shears at 60° C. while super skim milk did not show an initial

fall, and this suggests that the fall in viscosity is due to some change in the fat fraction induced by the shearing.

SUMMARY

1. The viscosity temperature coefficient of homogenized milk has been determined with an estimated accuracy of 1%.

2. The effect of temperature on the viscosity has been investigated in some greater detail.

The author wishes to acknowledge the help he received from Dr R. Aschaffenburg, and particularly from Dr G. W. Scott Blair in whose department the work was carried out, and from the Express Dairy Co. Ltd., who supplied the samples.

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637.127.1

439. THE RATE OF CREAMING OF RAW COWS' AND BUFFALOES' MILK

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(With 2 Figures)

INTRODUCTION

The rate of creaming of milk as measured by the depth of the cream-layer formed on the surface is of major importance in some countries where cows' milk is distributed in glass containers, since many consumers regard cream volume as an index to the richness of the milk.

The importance of this property has long been recognized in the milk industry, and many studies have been carried out on the creaming of cows' milk. Dahlberg & Marquardt (1) and Dunkley & Sommer (2) have given extensive bibliographies. None of these studies, however, referred to the creaming of buffaloes' milk, despite the fact that this milk is very popular in several countries, for example in India and Egypt.

The scarcity of literature on the creaming properties of buffaloes' milk is probably due to:

(1) The absence of yellow pigments in the fat of buffaloes' milk renders the observation of the cream-layer volume (C.L.V.), particularly in the first stages of creaming, more difficult than in the case of cows' milk.

(2) The very slow rate of creaming of buffaloes' milk at low temperatures does not allow of the formation of an appreciable C.L.V. in the interval between milk production and distribution. Thus, unlike the consumers of milk in northern countries, the consumers of buffaloes' milk are not accustomed to regard the depth of the cream layer as of great importance.

(3) While at higher temperatures the creaming of buffaloes' milk proceeds quite rapidly, the keeping quality of the milk becomes short and hence the practice of boiling the milk is generally adopted. This process either reduces or destroys the C.L.V. so that again the consumer is accustomed to the absence of a cream-layer.

However, in the manufacture of *Kareish* cheese (3), one of the most popular types of soft cheese in Egypt, the creaming ability of milk has a marked influence upon the quality of the cheese produced from it since *Kareish* is made from the skimmed milk remaining after the gravity-separation of cream from raw milk. Such skimmed milk varies in composition according to the chemical, physical and bacteriological properties of the original milk, whilst such variable factors as temperature and duration of creaming also influence the composition of the final product.

EXPERIMENTAL

In this work the creaming power of milk was determined by both the rate of formation of the C.L.V. and by estimation of the fat percentage of the skim-milk.

The technique adopted was as follows. Samples of bulk milk were taken from the

Faculty's herds of the two types of animals, and mercuric chloride was added to the milk in the proportion of 2 ml. of 5% solution to 1 litre of milk to act as a preservative. It had previously been shown that neither the addition of mercuric chloride in this proportion nor formalin (1 ml. to 1 litre of milk) influenced the creaming process.

The C.L.V. was measured by placing 100 ml. portions of milk to which the preservative had been added in 100 ml. graduated cylinders of similar diameters. Duplicate samples were set to cream at room temperature for which an average value of 23·5° C. (74° F.) was recorded, and duplicates of similar samples were placed in a refrigerator at 10° C. (50° F.). The duration of the test varied with the temperature of creaming. In the case of milk creamed at room temperature, the maximum C.L.V. was observed before the third day in both kinds of milk, and therefore, these samples were creamed for 3 days only. Samples kept in the refrigerator were creamed for 10 days because the creaming of buffaloes' milk was much retarded at low temperature.

Skim milks were withdrawn by means of special creaming tubes, and their fat contents were estimated. These tubes measured 30 cm. in length and 35 mm. in diameter; on the tapered end of each of these was fitted a piece of rubber tube and a screw clip. Milk 1·5 cm. below the level of the cream layer was regarded as skim milk. The percentage of fat in the skim milk was estimated by the Gerber method as specified by the B.S.I. (4) When the milk was creamed at room temperature, determinations were made at 24 hr. intervals; when creamed in the refrigerator, determinations were undertaken after 1, 3 and 10 days respectively.

RESULTS

The C.L.V., which is a direct percentage of the volume of cream separated from 100 ml. of milk, is given in Table 1, and in Fig. 1 the values are plotted against the creaming time. The data in Table 2 (graphically expressed in Fig. 2) represent the figures obtained for the specific cream volume (S.C.V.). This quantity is obtained by dividing the C.L.V. by the percentage of fat in the milk. The S.C.V. provides a useful basis for the comparison of the creaming abilities of milks containing different percentages of fat. Table 3 shows the percentages of fat in the skim milk.

Table 1. *The effect of time and temperature of creaming upon the cream-layer volume of cows' and buffaloes' milk*

Exp. no.	Kind of milk	Fat in milk (%)	Room temperature Cream-layer volume of milk held for 1 day	2 days	3 days	Refrigerator Cream-layer volume of milk held for 1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days
1	Cows'	3·75	6·5	6·5	6·0	10·5	10·0	9·7	9·0	9·0	9·0	9·0	9·0	9·0	8·7
2	Cows'	5·80	11·0	11·0	11·0	19·5	19·0	17·5	17·0	16·0	16·0	16·0	16·0	15·0	14·0
3	Cows'	3·60	5·5	6·0	6·2	12·5	12·0	11·0	11·0	11·0	11·0	10·5	10·5	10·0	9·7
Av.	Cows'	4·80	7·7	7·8	7·7	14·1	13·7	12·8	12·3	12·0	12·0	11·8	11·8	11·5	11·0
1	Buffaloes'	6·85	10·0	10·2	10·0	5·7	8·7	11·5	11·7	12·0	12·5	12·5	13·0	13·0	13·0
2	Buffaloes'	8·50	12·5	14·0	14·0	—	—	14·5	16·0	17·0	17·5	17·5	18·0	17·5	17·0
3	Buffaloes'	6·50	10·0	10·5	10·5	3·7	7·0	10·0	11·0	11·5	12·0	12·5	12·0	12·0	12·0
Av.	Buffaloes'	7·30	10·8	11·6	11·5	4·7	7·8	12·0	13·0	13·5	14·0	14·4	14·3	14·0	14·0

Table 2. *The effect of time and temperature of creaming upon the specific cream-volume of cows' and buffaloes' milk*

Exp. no.	Kind of milk	Fat in milk (%)	Room temperature Specific cream volume of milk held for 1 day	2 days	3 days	Refrigerator Specific cream volume of milk held for 1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days
1	Cows'	3·75	1·74	1·74	1·60	2·80	2·66	2·60	2·40	2·40	2·40	2·40	2·40	2·40	2·32
2	Cows'	5·80	1·90	1·90	1·90	3·40	3·30	3·00	3·00	3·00	2·80	2·80	2·80	2·60	2·40
3	Cows'	3·60	1·50	1·70	1·70	3·50	3·30	3·20	3·20	3·20	2·90	2·90	2·80	2·70	2·60
Av.	Cows'	4·80	1·71	1·78	1·67	3·23	3·09	2·93	2·86	2·86	2·70	2·70	2·67	2·57	2·44
1	Buffaloes'	6·85	1·46	1·50	1·46	0·84	1·28	1·68	1·70	1·75	1·82	1·82	1·89	1·89	1·89
2	Buffaloes'	8·50	1·50	1·60	1·60	—	—	1·70	1·90	2·00	2·10	2·10	2·10	2·00	2·00
3	Buffaloes'	6·50	1·60	1·60	1·60	0·60	1·00	1·60	1·70	1·80	1·80	1·90	1·80	1·80	1·80
Av.	Buffaloes'	7·30	1·52	1·57	1·55	0·72	1·14	1·66	1·77	1·85	1·97	1·94	1·93	1·90	1·90

Table 3. *The effect of time and temperature of creaming upon the percentage of fat in the skim milk*

Exp. no.	Kind of milk used	Fat in milk (%)	Fat percentage in skim milk: Room temperature 1 day	2 days	3 days	Refrigerator 1 day	3 days	10 days
1	Cows'	3·75	1·60	0·80	0·55	0·75	0·40	0·12
2	Cows'	5·80	2·00	0·90	0·60	1·00	0·50	0·20
3	Cows'	3·60	1·50	0·80	0·60	0·80	0·40	0·10
Av.	Cows'	4·80	1·70	0·83	0·58	0·85	0·43	0·13
1	Buffaloes'	6·85	1·80	1·20	0·70	3·60	2·10	0·30
2	Buffaloes'	8·50	2·10	1·40	1·00	—	2·60	0·50
3	Buffaloes'	6·50	1·90	1·20	0·75	3·80	1·90	0·40
Av.	Buffaloes'	7·30	1·93	1·27	0·82	3·70	2·20	0·40

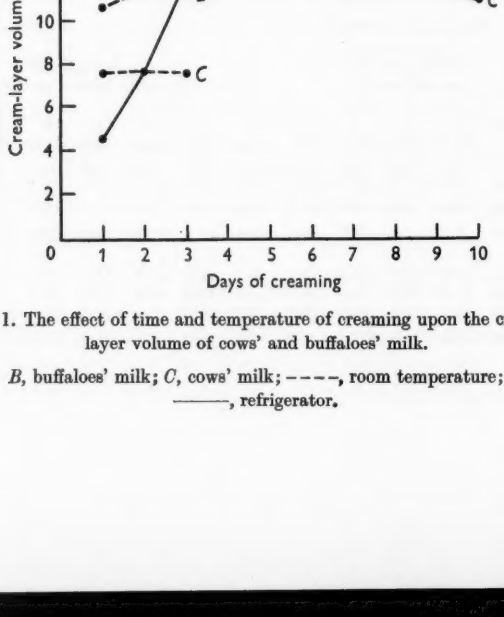

Fig. 1. The effect of time and temperature of creaming upon the cream-layer volume of cows' and buffaloes' milk.

B, buffaloes' milk; *C*, cows' milk; – – – –, room temperature; ———, refrigerator.

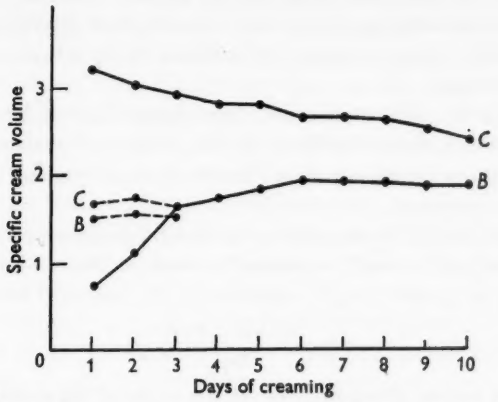

Fig. 2. The effect of time and temperature of creaming upon the specific cream volume of cows' and buffaloes' milk.

B, buffaloes' milk; *C*, cows' milk; – – – –, room temperature; ———, refrigerator.

DISCUSSION

The results of the tests on the creaming properties of cows' milk are in agreement with those reported by numerous other workers and may be summarized as follows:

(1) Milk gave a greater C.L.V. when creamed in the refrigerator than when creamed at room temperature, the measurements being commenced after 24 hr.

(2) The C.L.V. reached its maximum in refrigerated milk in less than 24 hr., while a longer time was required for maximum creaming when this was carried out at room temperature. A gradual decrease of the C.L.V. from the maximum took place when the milk was left for a further period of time. (This phenomenon has been attributed mainly to the closer packing of the fat globules which accompanies their rise and concentration in the upper part of the cream layer.)

(3) The C.L.V. varied from one sample to another and bore no relation to either the percentage of fat, or the type of milk used.

(4) The percentage of fat in the skim milk decreased with longer creaming time, this decrease being at first rapid but soon becoming slow. After the same length of time, the skim milk obtained from milk creamed in the refrigerator contained less fat than similar milk creamed at room temperature.

When buffaloes' milk and cows' milk were creamed under the same conditions, the results obtained in this study indicated that:

(1) Buffaloes' milk creamed in a very similar way to cows' milk with regard to the last three points.

(2) The fat percentage of buffaloes' skim milk was invariably higher than that of cows' skim milk when both were estimated after the same creaming time.

(3) While the maximum C.L.V. was greater at both temperatures in buffaloes' milk, the maximum S.C.V. was higher in cows' milk.

(4) The effect of creaming temperature on buffaloes' milk was markedly different from that on cows' milk. Thus, the rate of creaming of buffaloes' milk was very slow at refri-

gerator temperature; the maximum C.L.V. was not reached until the sixth or seventh day whilst at room temperature the maximum C.L.V. was observed after 48 hr. In other words, heat is required to effect rapid creaming of buffaloes' milk, whereas cows' milk creams better at low temperatures.

In order to explain the differences which have been observed between the creaming properties of cows' and buffaloes' milk at the two ranges of temperature used in these experiments, the primary factors which are known to be responsible for the creaming of cows' milk must be considered.

The rising of cream in milk is primarily due to the difference in specific gravity between the fat and the serum, and is usually expressed in terms of Stokes's law defining the rising of a sphere in a fluid of greater density according to the standard formula

$$V = \frac{2r^2 (ds - df) g}{9\eta},$$

where ds = density of serum, df = density of fat, r = radius of fat globule (cm.), g = force of gravity (980 dynes), η = absolute viscosity of liquid (poises) and V = rising velocity.

In their investigations on the creaming of milk Van der Burg (5), Rahn (6) and Van Dam & Sirks (7) found that the rising velocity of individual fat globules in milk was in agreement with Stokes's equation. The actual values for individual fat globules reported by these investigators, however, were realized to be much too low to explain the rapidity of formation of the cream-layer. They concluded that the discrepancy must be due to the accelerating effect resulting from the aggregation of the fat globules in milk during the creaming process. The work of Troy & Sharp (8) proved this was the case and their results were fully supported by Dahlberg & Marquardt (1).

In contrast to the voluminous literature on cows' milk, few contributions have been made to the physical chemistry of buffaloes' milk which might be extended to the process of creaming. Zein-El-Abidine (9) has reported that:

(1) The difference between the density of the serum and the fat of the same milk was greater in buffaloes' than in cows' milk.

(2) The diameter and the number of the larger fat globules were greater in buffaloes' than in cows' milk.

(3) Similar viscosity values were obtained for the separated milk of both cows and buffaloes (these values being estimated at 20° C.).

(4) Fat globules in buffaloes' milk showed no tendency to aggregate even when the milk was left for 3–4 hr.

Factors (1) and (2) above would be expected (from Stokes's equation) to favour greater creaming power in buffaloes' than in cows' milk. Since the viscosity is similar in the two milks, ds of Stokes's equation has the same relative effect on the velocity of creaming of both types of milk. Factor (4), i.e. the absence of cluster formation in buffaloes' milk, would suggest a possible explanation for the slower rate of creaming of buffaloes' milk. The writer's experiments, however, would indicate that on warming, creaming of buffaloes' milk proceeds at a much more rapid rate than would be expected for individual fat globules as calculated from Stokes's equation.

Thus, it is highly probable that clustering of fat globules takes place in buffaloes' milk as it does in cows' milk. In the case of buffaloes' milk the effect of heat on aggregation is operative at a higher minimum temperature, but once this minimum has been reached, the temperature coefficient is higher than for cows' milk. This explanation appears to the

writer to be a more probable one than that suggested by Troy & Sharp (8) in connexion with slowly creaming cows' milk, namely 'that the fat was in such a clumped condition that the cream layer occupied almost the entire volume of the milk and was therefore overlooked. Upon warming such milk the customary shrinkage in the depth of the cream layer probably occurred and the resulting cream layer was then recognized.'

Although it cannot be assumed that work on the creaming properties of cows' milk is necessarily directly applicable to other types of milk, the following information may prove of interest in connexion with creaming of buffaloes' milk:

(1) Marcas (10) mentioned that, in general, slowly creaming milk was richer in fat and total solids than ordinary milk. He also found more fat in the skim milk of the slowly creaming milk.

(2) Henseval (11), Marcas (12) and Hammer (13) found that samples of milk which failed to give a satisfactory cream line under standard conditions, gave a satisfactory cream line at higher temperature.

(3) Babcock (14) observed that, in general, retarded creaming gave deeper cream-layers with greater losses of fat in the skim milk.

(4) Woll *et al.* (15) regarded the normal creaming of milk to be due to the presence of clusters of fat globules and its failure to cream under some conditions to be due to the absence of such clusters.

These investigations suggest an analogy between the normal creaming of buffaloes' milk and that of cows' milk under abnormal conditions.

SUMMARY

A preliminary investigation was carried out on the effects of two ranges of temperature (room and refrigerator) on the creaming properties of cows' and buffaloes' milk. The results reported show that:

(1) While refrigerator temperature favoured the creaming of cows' milk, it greatly retarded the creaming of buffaloes' milk. This retarding effect was associated with a smaller cream-layer volume and a greater percentage of fat in the skim milk.

(2) At room temperature, the creaming of buffaloes' milk proceeded much more rapidly, suggesting that fat clumping was probably being induced.

(3) For both kinds of milk, when creamed at room temperature, the maximum values of the cream-layer volume and the specific cream-volume were found to be less than when creaming occurred in the refrigerator: reverse temperature relationships were observed in connexion with the maximum fat content of the skim milks.

In conclusion, it may be added that the observed variations in the cream-layer volume, and the fat content of the skim milks resulting from creaming at the two different temperature levels and for the varying lengths of time, would clearly result in some variability in any cheese made from such milk. This would particularly affect the yield, quality, composition and nutritive value of the cheese.

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REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION A. PHYSIOLOGY OF DAIRY CATTLE

PART II. PHYSIOLOGY AND BIOCHEMISTRY OF RUMINATION

Studies of rumination have now reached the interesting stage where an attempt at synthesis of empirical data may profitably be made without too much reliance on unsupported hypotheses. The modern researches stimulated by the approach of the recent World War, and actively continued since in many countries, have been a vindication of the inspired guess known as the hypothesis of Zuntz(1), who suggested that the abundant bacterial flora of the rumen were beneficent symbionts rather than mere commensals. This beneficent symbiosis is probably due to the age-long adaptation of ruminants to a diet rich in cellulose, of which a cow may digest more than 60% of its large intake(2). Ruminal bacteria perform many functions of value to their host. They bring about the digestion of cellulose, a process for which the host animal has no appropriate enzymes. They enable the host to cover a not inconsiderable proportion of its amino-acid requirements by means of such simple nitrogen compounds as ammonium salts, glycine or urea(3). Finally, they render the host independent of exogenous supplies of many of the water-soluble and at least one of the fat-soluble vitamins (vitamin K(3)).

These are all major advantages which have probably ensured the survival of the ruminant during the geologically recent vicissitudes of the earth's climate. There are also certain minor biochemical advantages in the possession of a rumen with its abundant microflora. Thus phytin, which is abundant in all seeds and which in non-ruminants is rachitogenic, is hydrolysed completely into non-toxic products in the rumen(4). Defatted soyabean (so-called soyabean oilmeal) unless heat-treated to deactivate the antitrypsins which it contains is toxic to the rat(5). The ruminant, however, tolerates it well.

Rumination has, of course, its disadvantages too. Thus a ruminant is not as efficient a digester of its preferred diet as is, say, a carnivore, of its preferred diet. With bacterial aid it can digest cellulose which the carnivore can hardly digest at all, but even with bacterial aid its digestion is comparatively slow, and a large fraction of the cellulose normally passes through the alimentary tract undigested. The ruminant's great merit from the economic viewpoint of the animal husbandman is that it digests a diet so rich in cellulose as to be useless to other domestic animals(2, 6, 7). It will tolerate far more crude fibre (cellulose) in its diet than will chickens, pigs or even rabbits(2). In comparison with the pig or the dog, the ruminant has a large appetite which is a necessary consequence of its large intake of food of low digestibility.

To facilitate bacterial digestion the ruminant has a large 'fill'(8), that is to say, the ratio of the weight of food and food residues in the animal at any time to the weight of the animal's living tissues is large. This is due to the delay of the food, during its passage through the animal, in the rumen, the caecum and the colon, in all of which chambers bacterial fermentations are active. Thus the ruminant carries more food about with it than animals not so efficient as bacterial digesters, and several days may elapse between ingestion of a meal and its subsequent elimination(9), in spite of continuously intermittent defaecations.

Another penalty of bacterial digestion is, according to Brody(10), the large heat increment which results after eating, for the animal has little or no control over this portion of the calorific value of the food. Thus the specific dynamic action in a carnivore is chiefly due to the heat liberated in the liver in deaminating surplus amino-acids resulting from digestion, while in a ruminant, in addition to this, there is the heat generated by fermentations in those parts of the gut where food is delayed. Such heat, though a benefit to the host in cold weather or to an animal on a main-

tenance ration may, in an animal in a hot environment, or in a hyperthyroid animal (11), or in a heavy yielder of milk, cause a heat burden which the host finds difficult to dissipate.

Thus in the ruminant much energy is liberated from the food by fermentation before it reaches the tissues proper, and a heavy energy tax is also involved in mastication and rumination. Other advantages and disadvantages of rumination will be referred to in later sections of this review.

Among reviews of rumination may be mentioned those by Elsden & Phillipson (12), Baker & Harriss (13), McNaught & Smith (14), Kon & Porter (15) and Phillipson (16). Johansson & Sarles (17) have reviewed the biological importance of intestinal micro-organisms.

GENERAL

Sets of monozygous cattle twins have been carefully collected both in Sweden and in New Zealand (18), and a quite considerable set of experiments on these has been published from Sweden. Bonnier, Hansson & Skjervold (19), in a paper on their experiments with such monozygous twins, draw the following conclusions:

(1) A given feeding intensity may not be sufficient for the attainment of the hereditary ceiling for a given character.

(2) If the feeding intensity is too high again the ceiling may not be reached. They quote experiments on milk yields to which these two generalizations applied.

(3) When the yields of a set of cows liberally rationed are compared with those of their identical twin sisters stringently rationed a marked correlation between the yields of twins is found. Hence, say these authors, the popular belief that to underfeed a high yielder is worse than overfeeding a low yielder is not supported by these results.

Ritzman (20) found wood yeast as good a milk producer in cows as soyabean. This observation has obvious economic possibilities in countries where timber with its large quantities of useless branches is abundant.

Tribe (21) has studied the grazing habits of sheep in much the same way as Johnstone-Wallace & Kennedy (22) studied cattle. He finds that sheep graze for 9½ hr. and rest for 13 hr. out of the day.

Seath & Miller (23), in studying cows in Louisiana, found that during summer more time was spent grazing by night than by day.

In the studies by Ragsdale, Brody, Thompson & Worstell (24) it was found that heat depressed both milk yield and food intake, the former more than the latter. Both food intake and milk yield rapidly revived when the temperature was restored to normal.

Fat-soluble materials readily pass into milk fat. It would be well if every new insecticide or weed killer were tested for power to reach the milk. Thus DDT from contaminated hay, according to Smith, Hoskins & Fullmer (25) and Shepherd, Moore, Carter & Poos (26), is secreted in milk. Phillipson (27) has shown that the flow of digesta from the rumen is intermittent. Hitchcock, Karvonen & Phillipson (28) have found abomasal pH in lambs to be increased by insulin. Paloheimo, Salonoja & Aïjälä (29) have measured the capacity of the bovine gut.

In ruminants oxalic acid is toxic as it is in rats and humans; but the reason for its toxicity in the ruminant is, according to Talapatra, Ray & Sen (30) peculiar. They have shown that excess oxalate in the ruminant, like excess citrate in the human, acts by changing the animals acid-base balance. They were studying the well-known toxicity of rice straw when fed to cattle, and they showed that this toxicity is due to its oxalate content. They partitioned the oxalate in the straw into insoluble (CaC_2O_4) and soluble (mainly $K_2C_2O_4$). The CaC_2O_4 they found traversed the gut of rat or bullock unchanged, thus showing that the lack of value of such a food as spinach to the rat is due to its having no calcium in excess of that which is present in CaC_2O_4. With rice straw or napier or guinea grass however, in the rat, but not in the bullock, the soluble calcium from other foods in the diet can be rendered unavailable by the $K_2C_2O_4$ which is present in much greater concentration than the CaC_2O_4, so that a negative calcium balance results. A negative calcium balance also occurs in the bullock when $K_2C_2O_4$ or rice straw is fed. In this instance, however, the negative calcium balance is not due to precipitation of CaC_2O_4 in the gut but to alkalosis. The conversion of oxalate to carbonate in the rumen leaves an excess of alkalinity due

to the potassium ion and, in readjusting its acid-base balance, the animal passes into negative calcium balance. This interpretation also explains an earlier observation of Richards, Godden & Husband(31), who found negative calcium balances in ruminants ingesting potassium citrate. These authors attributed the negative balance to inhibition of absorption of calcium by potassium in diets of high K:Na ratio. Talapatra *et al.*(30) showed that $Na_2C_2O_4$ also produces a negative calcium balance, thus showing that the toxicity of potassium oxalate in rice straw is a specific property neither of the potassium ion nor the oxalate ion but of the disturbance of the bullock's acid base balance resulting from the production of carbonate from oxalate in the rumen. Presumably half the carbon of the oxalate ion would go to methane in the rumen but Talapatra *et al.* did not investigate that point.

It has long been known that under certain conditions freshly dug mangolds or oat hay, both of which contain nitrate, may be extremely toxic to ruminants. Nitrate is peculiarly metabolized by the ruminant. Sapiro, Hoflund, Clark & Quin(32) have made an interesting study of the fate of nitrate in the rumen and have shown its logical connexion with earlier work done in South Africa in 1933(33). Experiments with rumen contents incubated *in vitro* showed that NO_3 disappeared more quickly from the rumen of sheep on lucerne than from the rumen of those on hay. Nitrate is rapidly changed to nitrite by rumen contents, and nitrite in turn is further reduced probably to ammonia. The rate of disappearance of nitrate is an index of the vitality of the rumen micro-organisms. Both nitrate and nitrite in rumen contents disappear more rapidly in the presence of added glucose than its absence. The lesion produced in sheep by nitrate ingestion is methaemoglobinaemia, and studies with eight sheep, four on grass hay and four on lucerne hay, showed the effect of sucrose in preventing this lesion. The authors suggest that nitrate, in small concentrations in food of ruminants, could be incorporated into the animal's body protein after reduction to ammonia in the rumen.

At this point it is convenient to mention that the possibility exists that some nitrogen, perhaps only traces, may conceivably be gained by rumen bacteria from the atmosphere. Toth(34), who was first to demonstrate the fixation of atmospheric nitrogen by green flies, has isolated nitrogen fixers from the faeces of cows, sheep and horses. He has not shown whether these bacteria can or do fix nitrogen when in their hosts, but it would not be surprising if tracer experiments demonstrated such fixation.

It has been disputed whether elementary hydrogen is ever an important constituent of rumen gases. Mangold quotes people who reported its presence, but Lugg(35) could not find it. Recently, however, Pilgrim(36) in Australia has found conditions in sheep with a rumen fistula, under which there is a copious evolution of this gas. On refeeding after starvation or on refeeding after emptying the rumen through the fistula, H_2 production was maximal $\frac{1}{2}$–1 day after refeeding, and could become sixteen times as fast as methane production. Three days after refeeding no hydrogen could be demonstrated. The author suggested that two bacterial species were involved, one producing the hydrogen and the other using it to reduce CO_2 to CH_4. We have here further evidence of the anaerobic conditions prevailing in the rumen where, according to McNaught, Owen & Smith(37), all the ionic iron appears to be in the ferrous state, and where growth of rumen bacteria *in vitro* occurs every bit as well in the presence of added thioglycollic acid, the substance routinely used by bacteriologists for producing anaerobiosis. Ruminants which have sudden access to lush and abundant pasture may suffer from bloat (tympanitis). Bloat(38) is a hazard peculiar to ruminants, and is generally believed to be due to some fault in the process of belching. One school of thought attributes bloat to the presence of toxic substances in the food (39, 40, 41), but experiments in South Africa of Quinn and his colleagues make this unlikely(3). Another school of thought favours the saponin theory which supposes that the inability to belch is due to soap-like substances, whose presence in leaves has been demonstrated. These saponins prevent the gases CO_2 and CH_4, produced by rumen fermentations, from forming large enough bubbles to be expelled by belching during rumination so that the rumen becomes filled with an intractable emulsion(42). Such emulsions have been observed by the reviewer in rats which failed to refect and therefore died when placed on a refective diet containing raw potato starch. Weight is lent to the emulsion theory by the successful treatment of bloat with such substances as

turpentine and 'tympanol'(43) which have the power to break emulsions by raising surface tension, presumably by dissolving saponins. Rumen gas formation has been further studied by Cole & Kleiber(44).

The production of fatty acids as a result of bacterial activity in the rumen makes the metabolism of ruminants very much different from that of other mammals, and also has far-reaching effects on its general metabolism. Thus the cow's mammary gland can assimilate acetic acid more readily than glucose, and it has been demonstrated by Popják, Folley & French(45), using radiocarbon, that the peculiarly large concentration of lower volatile fatty acids in cows' milk is, in part and perhaps entirely, due to the acetic acid and other simple acids in the cow's blood. This lends further support to the hypothesis that the positive correlation of the R.M. value of the milk of any species of mammal with its herbivorousness is due to the greater extent of acidogenic fermentation in the larger and more complicated gut of the more herbivorous animal(3).

A consequence of the rapid fermentation which occurs after feeding in the ruminant is that in the ruminant after heavy feeding metabolic conditions are very different from those produced in a non-ruminant. In a non-ruminant there is the well-known sharp rise followed by a fall in the blood glucose. In the ruminant (McClymont(46)) there is, in addition, a rise of blood acetic acid. It can be calculated from the data of Barcroft(47) that the metabolizable energy of acids coming from the rumen is about 40% of a sheep's basal metabolism. McClymont has shown by measuring arterio-venous differences that this blood acetate of the cow is largely taken up by the mammary gland. For a critical and informative account of current researches on the role of lower fatty acids such as acetic in mammary secretion the reader is referred to the review by Folley(48). Folley & French(49, 50) have compared the metabolism of mammary tissue slices from cow, goat, sheep, rabbit and rat, and have shown that when glucose is present the ruminant tissues utilize acetate much better than do the non-ruminants tissue. Popják *et al.*(45) have recently adduced evidence for the production of lower fatty acids from acetic acid by using radioactive tracers. These studies show that when acetic acid marked on the carbon of the carboxyl group is introduced into the goat, it appears in the milk. The fatty acid moieties obtained from the butterfat of these animals were (in order of increasing content of marked carbon): (*a*) higher saturated fatty acids, (*b*) higher unsaturated fatty acids, (*c*) lower unsaturated fatty acids, and (*d*) lower volatile fatty acids. An important point in their researches is that the marked carbon content of the milk fat was greater than that of the liver fat, so that the role of the liver in assimilating acetic acid could only have been a minor one. These chemical and physiological observations of McClymont, and of Folley and his colleagues, show that the ruminant's mammary gland has developed its enzyme systems in adaptive relation to the acetic acid which results from the metabolism of its alimentary symbionts.

The importance of lower volatile fatty acids in ruminants has led to a stimulation of interest in their metabolism in other animals, and it has become obvious that acetic acid is metabolized by most tissues. Rabbit heart, rat liver, rat artery wall, the head of the living sheep and the mammary gland can all use it. Since Phillipson and his colleagues demonstrated the absorption of acetic, propionic and butyric acid into the portal circulation, further interesting observations on the metabolism of acetic acid have been made at Cambridge. McClymont(46) has found that 40–80% of the blood acetic acid is removed by the mammary gland. McClymont(46), by infusing acetic acid into the rumen or venous system and by giving oxytocin to ensure thorough emptying of the udder, attempted to increase the small Reichert-Meissl value(51) of the starved animal. His attempt failed, but in view of the results of Folley & French and of Popják *et al.*, already quoted, a repetition of McClymont's experiment using a mixture of acetate and glucose might conceivably meet with success, for glucose facilitates the utilization of acetic acid by mammary slices *in vitro*. Reid(52) has confirmed Elsden's(53) observation that 90% of the lower volatile fatty acids in sheep's blood is acetic. He found, as had McClymont for the cow, that the lower volatile fatty acid is much greater (up to 10 mg. 100 ml.) in sheep's blood than in the blood of non-ruminants. Arterio-venous differences showed that much of the acetic acid in the carotid artery was removed by passage through the tissues of the head, and that the quantity removed depended on the arterial content of it. The small concentration of propionic acid in blood shows how efficiently the liver

removes it from the portal blood. Propionic acid being a 3-carbon compound passes into the carbohydrate cycle in the liver, and in keeping with this Phillipson & Hitchcock (54) and Jarret (55) found that propionic acid is excreted as glucose by the phlorizinized dog. Jarret also found that sodium propionate can quickly restore the blood sugar of the insulinized sheep. Thus propionic and acetic, though both produced by bacterial action in the rumen, suffer quite different fates in the host's tissues. Gray (56, 57) has measured the rate of disappearance of mixtures of lower fatty acids placed in the emptied rumen of sheep. One-third of a mixture of the sodium salts of acetic, propionic and butyric acids disappeared in 6 hr. When the animal was fed, propionic and butyric rapidly disappeared. When a mixture of propionic and acetic acids buffered to pH 6·5 with NaH_2PO_4 and $NaHCO_3$ was added, propionic was absorbed faster than acetic. Hence the low proportion of propionic usually present in rumen contents is probably due to more rapid absorption. It may be convenient to mention at this point that the mixture acetic + propionic + butyric acids is ingested preformed in the cow's diet when she is eating silage. McNaught (58) in this laboratory has partitioned acetic, propionic and butyric by the procedure of Moyle, Baldwin & Scarisbrick (59) in the juice of good quality silage. Propionic acid, contrary to the prevalent assumption, is present.

KETONAEMIA

Ketonaemia is a disability from which cattle and other animals suffer, but its causes in ruminants are still obscure. Its possible connexion (see previous biennial review) with vitamin A deficiency does not seem to have been confirmed, but that it is a dietary disorder is very likely. Boddie (60) has found that ketonaemia in cows is associated with a low blood sugar (30–35 mg./100 ml.) and that it is much more prevalent in dairy than in beef cows. In dairy cows kept indoors ketosis increases during the winter. Ketones, he finds, are normally produced in the rumen of the cow and are absorbed and utilized by the cow. Incubations *in vitro* of late winter rumen contents, with such foods as turnips, produces substantial quantities of ketones. In samples of rumen contents obtained by stomach pump, ketones can readily be demonstrated and they are probably products of bacterial fermentation. It seems then that the aetiology of ketosis in the ruminant may be different from its aetiology in non-ruminants. Observations of the occurrence of ketosis in cattle and sheep can be explained on the basis of observations on non-ruminants (Sampson (61)), but there is a growing tendency to implicate the rumen. Thus Boddie (60) has shown ketones to be produced in it, while Hoflund & Hedstrom (62, 63) state that the ketosis which occurs in good yielders of milk is due to failure of the rumen fermentation of cellulose to provide sugar fast enough. Any change of diet, such as giving too much protein, which retards rumen fermentation is conducive, say these authors, to ketosis. Henderson (64) points out that in U.S.A. ketosis is common in cobalt-deficient areas. Of twelve cows which were diagnosed as suffering from ketosis nine improved when given cobalt. Dye & Candless (65) point to the possible connexion between ketosis in ruminants, their habitually low blood sugar and the large amounts of lower fatty acids which circulate in their blood. Schultz, Smith & Lardy (66) studied the possible origin of ketones in cattle blood from lower fatty acids and found butyric and higher acids effective in producing ketone bodies. Acetic, propionic, lactic, succinic, lauric and oleic were ineffective. McBarron (67), in Australia, successfully treated ketosis of dairy cattle on pasture with molasses and yeast.

By 60% underfeeding 10 days before calving and by 30–50% underfeeding thereafter, Shaw (68) produced ketosis in cows, with blood ketones ten times their normal value and a 50% reduction in blood sugar. Realimentation restored the blood picture within a few days. Blood plasma carotene and vitamin A were unaffected and vitamins A, B or B complex therapy did not improve the ketosis. Treatment recommended is intravenous glucose with calcium gluconate if milk fever is likely. In diagnosis blood ketones should be estimated, as urinary ketones may change for other reasons. The use of molasses before and after calving is recommended as a prophylactic. Schultz *et al.* (66) have tested various fatty acids as sources of ketones in the blood of goats. Butyric, caproic, capryllic and capric generated ketones in the blood when placed in the rumen, but

acetic, propionic, lauric and oleic did not. Corn oil was not ketogenic. When acetic and butyric were injected intravenously only the latter produced ketosis.

Interest in the feeding of urea to cattle and sheep still continues. Dinning *et al.* (69) have confirmed the observation of McDonald (70) that urea placed in the rumen appears as ammonia in the portal blood. Rises of urea in the systemic blood similar to those found by Owen, Smith & Wright (71) were also observed. Dinning *et al.* (69) found, as has been repeatedly found before, that urea is rapidly hydrolysed to ammonium carbonate in the rumen, and they also investigated the blood levels at which ingested urea became toxic by administering graded doses of urea to each of four steers as a drench. Steers, nos. 1–4, received 57, 116, 272, and 490 g. urea respectively. Ataxia developed with the three highest doses at 2·5 mg. ammonia-N in blood and of steers 2–4 death occurred at 4½, 6 and 7 mg. respectively. That it was the ammonia and not the urea *per se* which was toxic could be deduced from the fact that the blood urea of the animal which survived, steer no. 1, was about the same as that of animal no. 3. When mixed with concentrates the urea showed no sign of toxicity when 400 g. was given per day. This is about four times the dose fed by Owen *et al.* to milking cows (71). Briggs, Gallup, Heller & Darlow (72) reported positive nitrogen balanced on lamb rations containing urea, and the same group of workers (Briggs, Gallup, Darlow, Stephens & Kinney (73)) found urea as 25 or 50% of the nitrogen of the rations was satisfactory for maintenance or fattening. On a wintering ration, however, the steers refused a supplement containing 50%, but took that containing 25% urea. By comparison of the effects of the same supplements not containing urea, it was shown that urea increased the retention of nitrogen in both lambs and steers. The apparent digestibility of the constituents of the ration was unaffected by urea. Analysis of expired air of animals receiving urea demonstrated the absence of ammonia in it.

Many more studies (74, 75, 76, 77) have been made of urea as an extender of protein in sheep and cattle rations. Nearly all the studies show that urea has a definite but limited power to supplement the animal's protein supplies. The experiments of Bowstead & Fredeen (78) show that the method of mixing the urea with the ration and the composition of the ration have important effects on the palatability of urea rations fed to cows over a long period. In these experiments the importance of extra grain and molasses in the rations is made evident, for urea rations without these additions were consistently refused by certain of their cows but were accepted when the additions were made. In these experiments gradual addition of urea into a ration presented no advantage in respect of palatability.

From a 3-year wartime experiment in Sweden on seventy-two cows divided into four equal groups, one of which received urea, Ulvesli (79) concluded that the protein value of urea is about 40%. Cows in milk can utilize urea when they are receiving a ration poor in protein and containing sugar and cellulose but only traces of starch. From his experiment Ulvesli recommends that the minimum digestible crude protein requirement per kilogram milk corrected to 4% content of fat is 40–50 g. This is very similar in magnitude to the recommendations of Morris, Wright & Fowler (80).

Hamilton, Robinson & Johnson (81) have modified the previous generalization of Miller & Morrison (82), who said that all proteins tended to have the same biological value in sheep and that this was due to their protein being entirely converted to bacterial form before being digested in the abomasum and intestines. Hamilton *et al.* (81) obtained results consistent with Miller & Morrison's generalization, provided only diets containing less than 12% total protein are considered and provided at least one-sixth of the nitrogen is ingested as preformed protein. Under these conditions, urea, skim-milk (with or without extra cystine), gluten feed, or casein (with or without extra cystine), had the same biological value in paired feeding experiments with growing lambs. Linseed meal was, however, an exception in being better than urea in these experiments. This the authors attribute to a failure of the linseed to be readily attacked by rumen micro-organisms so that it passes out of the rumen and is digested in the ordinary way. Urea added to a ration already containing 12% protein is likewise unavailable, so that in these experiments the ration containing 16% protein with 63% in the form of urea was worse utilized than one with 11·4% protein and 46% in the form of urea.

Briggs *et al.* (72) showed that pellets containing 4% urea increased the digestibility and retention

of nitrogen from a basal ration of hay almost as well as did cottonseed meal, but the effect was much smaller when the supplements contained 8 or 12% urea. The effect of supplying a source of energy with urea is again shown in these experiments. These authors had found in an earlier experiment (73) with yearling steers that the same 4 and 8% pellets were about as good as cottonseed meal in aiding nitrogen retention. Only with the 12% pellets was a diminishing return noticeable. In a later experiment, Dinning, Briggs & Gallup (83) fed these rations simultaneously to lambs and steers, and found that the former were superior to the latter in using urea. In another experiment with cattle reported in the same paper, Briggs *et al.* (73) showed that when fed with a fattening basal ration of hay and ground corn the cubes containing 4 or 8% urea were somewhat better than cottonseed meal in respect both of digestibility of proximate constituents and of nitrogen retention. The same authors (Dinning, Gallup & Briggs (84)) have found marked variation of creatine excretion in cattle consuming food containing urea.

Lofgreen, Loosli & Maynard (85) have further investigated the function of sulphur-containing amino-acids in the metabolism of lambs. Six grade wethers were fed four rations in turn, each ration contained 10% of protein, 40% of which came from urea, or from urea plus 0·2% methionine, or from linseed meal, or from dried egg. The urea plus 0·2% methionine produced as good a nitrogen retention as did linseed meal, but not as good as did the dried egg. An effect of protein quality, independent of rumen bacteria, is indicated in these experiments. Watson, Kennedy, Davidson, Robinson & Muir (86) compared urea with casein as an improver of a basal ration poor in nitrogen. Casein was superior to urea in producing growth in beef calves and in producing growth of wool in sheep. Watson, Davidson & Kennedy (87) showed by giving urea containing N^{15} that the nitrogen therefrom finds its way into all the body protein which they tested, thus confirming Schonheimer's observations on the rat. Unfortunately, however, they did not determine the N^{15} in rumen bacteria or protozoa, so that the experiment adds nothing to our knowledge of the difference between ruminant and non-ruminant metabolism. The experiments of Briggs *et al.* (72) and those of Loosli *et al.* (85) seem to show that there is a limit to the power of rumen bacteria to supply their host with amino-acids. This may not necessarily be so, for Loosli, Williams, Thomas, Ferris & Maynard (88) have described an experiment in which three sheep and two goats were kept in positive balance of nitrogen for 10 days on a ration consisting of maize starch, maize sugar, cellophane, lard, urea, minerals and vitamins A and D in a ration containing no protein (except for traces in the maize starch and lard) and no water-soluble vitamins. The sheep gained 0·23 lb./day over 3 months on this ration. Hence, synthesis of amino-acids is, say the authors, obvious. In these experiments ten amino-acids were estimated microbiologically in food, faeces and urine. Samples of rumen contents obtained by stomach pump were also analysed. The amount in the diet alone was insufficient to allow growth, but the rumen contents showed a large increase in all of them. The loss of these amino-acids in the faeces and the urine was greater than their intake in the food, but much less than the amount in the rumen contents. These results seem to conflict with those of Dinning *et al.* (83), and would have been more convincing if the total rumen contents at slaughter had been analysed, and if the nitrogen balance had been investigated every 2 days for the whole period rather than for one final period of 10 days, when a false equilibrium may have been attained. Why the toxicity of excess of urea was not encountered in these experiments needs to be explained, as also does the source of sulphur for the methionine which must have been synthesized.

AMINO-ACID METABOLISM

In man, pig and dog the initial alkaline treatment of the food with saliva is very brief, and is followed by peptic digestion at a pH which may be as low as 1·5 (Møllgaard, Lorenzen, Hansen & Christensen (89)). Such a pH is an effective inhibitor of bacterial action, which does not subsequently supervene till the food has passed through the small intestine. Such fermentation as occurs in these animals is normally confined to the large intestine and rectum where it is progressively inhibited by resorption of water and, no doubt, of certain vitamins. As a source of macronutrients it is, however, negligible.

Digestion in the ruminant is different. There is no ptyalin in the saliva, which merely lubricates dry food and serves as a culture medium for the microflora of the rumen, containing as it does considerable phosphate. Thus the first process taking place in ruminant digestion is a fermentation in the capacious rumen. As a result of this fermentation organic acids are rapidly produced and the pH falls. The bicarbonate and phosphate in the saliva (90), which continually pours into the rumen as the cud is chewed, prevents the pH from becoming too acid and thus slowing the fermentation. Recent studies of the chemistry of ruminant saliva serve to emphasize its neutralizing function (91). Ever since the demonstration by Phillipson and his colleagues that acetic, propionic and butyric acids are readily absorbed into the ruminal veins (3), and hence into the portal circulation, the possibility of the similar absorption of other small molecules through the stratified epithelium of the rumen has been intriguing the minds of researchers. Thus McDonald (70) has produced evidence that ammonia is absorbed into the venous circulation of the rumen, and that some of it enters the rumen again in the saliva in the form of urea. Presumably, therefore, there is a nitrogen cycle from the rumen as NH_3 to the liver (where NH_3 becomes $(NH_2)_2CO$), then to the salivary glands, and so back to the rumen as urea. In McDonald's experiments his sheep were being maintained on hay, and McDonald showed that such nitrogen, reintroduced into the rumen as urea by the saliva, is essential for the efficient digestion of cellulose which occurs in sheep on a maintenance diet of pure hay. Earlier, South African field experiments had shown a similar phenomenon in demonstrating that fodder was better digested when supplemented by urea in sheep on a maintenance diet (3). Increased digestion of cellulose as a result of adding urea to the diet had also been found by workers in the U.S.A. (3). In the cycle mentioned above ammonia will, according to the observations of Pearson & Smith (3) and McDonald (92), result from the hydrolysis of proteins. This protein hydrolysis in the rumen facilitates microbial growth, and therefore indirectly benefits the host by providing the rumen bacteria with ammonia. In simple-stomached animals this extensive degradation of protein does not occur, peptides being the chief hydrolytic products. It is therefore conceivable that some diets will lead to much greater retention of dietary nitrogen in the animal with a simple stomach than in the ruminant, and Cuthbertson & Chalmers (93) have produced evidence supporting such a hypothesis. In pregnant and non-pregnant ewes on a low plane of nutrition they showed that a much greater percentage of casein was retained by the animal when the casein was introduced by fistula directly into the duodenum than when it was introduced by fistula into the rumen. When introduced by the former route a little of the casein might reach the rumen (Phillipson & Mitchell (94)), but the bulk of it would probably escape bacterial action. This, then, illustrates an additional possible disadvantage of microbial digestion, since it demonstrates how a protein may have a large biological value in a non-ruminant and a much smaller one in a ruminant. Thus the observations of Cuthbertson & Chalmers (93) are consistent with the theory of Miller & Morrison (82) that all proteins in sheep may tend to have the same biological value. However, in ruminants as in other animals, as the food intake increases, its rate of passage through the gut is accelerated. Such acceleration would result in the ruminant in a decrease of the ratio of bacterial to peptic-tryptic-ereptic digestion so that, as mentioned in the present writer's previous review (3), an animal producing a large milk yield and therefore necessarily heavily fed may be much less dependent on bacterial digestion than in a sheep on maintenance. This is probably why, in pre-war Germany the utilization of urea, ammonia and glycine absorbed into beet slices was so easily demonstrated in lambs but could not be demonstrated with cows (Krebs (95)).

The rat, the chick, the dog and human-being require sulphur in the form of methionine, though the sulphur may in part be supplied as cystine. Since the sheep can undoubtedly subsist on bacterial protein, and since the cow in milk can in part do likewise, it seems legitimate to infer that some sulphur in a simpler form than cystine can contribute to the ruminant's sulphur requirement. Thus Ferrando (96), in France, has kept sheep in positive sulphur balance on diets containing thioglycollic acid which was long ago shown to be of no use as a substitute for cystine in a rat's diet. Reed, Moir & Underwood (97) have presented indirect evidence that sheep obtain methionine microbiologically. McDonald (92,98) has shown that a sheep on a diet containing 82% of its protein as zein, can synthesize bacterial protein containing 7% of lysine. McDonald (92)

calculated from his experiments that the sheep could convert 40% of the zein in the diet into microbial protein. McDonald's experiments with zein have an added significance, for lysine is one of the amino-acids which the rat needs fully aminated, and is one to which Schonheimer and his colleagues attributed a leading role in vital processes(99). Recent experiments by Blaxter & Wood(100) give an indication of the amino-acid requirements of the cow's tissues, for they have shown that in the calf, on a semi-synthetic milk diet and too young for development of rumination, gelatine cannot produce a positive nitrogen balance as a skim-milk diet does. Presumably the pre-ruminant calf, like the rat, man, dog, pig, mouse or chicken needs tryptophan which Chanda & Kehar(101) have shown to be synthesized in the adult cattle-beast.

Branton, Bratton & Salisbury(102) found no difference in the semen production or fertility of bulls fed rations containing plant or animal proteins. The arginine in the rations, which might have been expected to have made a difference in view of its need for human spermatogenesis(103), was however not determined.

Dalgarno, Godden & McCarthy(104) studied the serum protein of the ewes and lambs described in the experiment of Thomson & Thomson(105). Ewes fed 1·9 lb. gross digestible nutrients per day had more protein in the blood serum than ewes receiving 0·9 lb. gross digestible nutrients. Ratios of albumin to globulin were, however, unchanged. There was a prepartum fall in one component of the electrophoretic diagram of the blood serum. This was tentatively attributed to a translocation of it to colostrum.

RUMEN MICROBIOLOGY

Pounden & Hibbs(106, 107, 108, 109), studying the way in which young ruminants can acquire their characteristic ruminal flora, found that a greater variety of bacteria develop in the rumen of inoculated as compared with uninoculated calves. In uninoculated calves protozoa do not develop at all. A normal facies of bacteria and protozoa develops only in calves specially inoculated or else depastured with calves reared in the normal way. Hibbs & Pounden(110) also found that carotenoids, vitamin A and ascorbic acid in plasma and in the liver were unaffected by rumen development. It is known that bacteria synthesizing carotenoids occur naturally in oceanic sediments and in crude petroleum residues, but no evidence has yet been found of carotenoid synthesis in the rumen.

The microscopic appearance of the contents of the rumen, and of other parts of the gut, has continued to be a field of research in South Africa, U.S.A., Holland, Britain and Canada. Baker & Nasr(111) and Baker & Harriss(13) in review articles, in which some of their own current work is mentioned, emphasize the difficulties of interpreting chemical changes occurring in the rumen if correlated changes in what they call the 'microbial facies' are ignored. They again stress the pitfalls in the paths of those who seek too early for pure cultures of rumen bacteria. The work of Pounden & Hibbs may have an application here, for the final test of a rumen organism must surely be that it performs as before when replaced in the rumen. Such a test is relatively simple when a pathogen is being replaced in its otherwise sterile habitat, say the blood, but in the rumen where there are already so many other lodgers biochemical changes cannot be easily attributed to any particular organism. It is for this reason that Baker & Nasr(111), Van der Wath(112) and Gall and others(113, 114, 115, 116, 117) attach such importance to microscopic analysis. Recent refinements of technique of microscopic observation are described in the paper by Baker & Nasr in which the changes which occur in the bacterial flora when cellulose and starch are being digested are described. Each of these substances tends to encourage a distinct microbial facies, as Van der Wath also noted. Baker & Nasr have described the microscopic changes suffered in the rumen by various forms of cellulose, such as herbage (old and young), cotton-wool, filter-paper, delignified paper pulps and cellophane. In the cellulosic materials, in which the original plant structure is still intact, enzymic cavities are eroded by the cocci responsible for its dissolution and these cavities are rendered very evident by a variety of techniques, particularly those making use of the pleochroism and birefringence of natural cellulose. Baker & Nasr and Van der Wath have investigated microscopically the fate of natural and dextrinized starch from various plants. Natural starches vary considerably in the rate of digestion by rumen bacteria. According to Van

der Wath, maize, peanut, rice and oat starch are most quickly digested in the rumen, wheat starch takes longer and potato starch longer still. Van der Wath's observations were uncomplicated by possible effects of rumen ciliates, for he defaunated his sheep with copper sulphate before his experiments. Van der Wath also demonstrated that the rumen bacterial facies is, as Baker has also found, dependent on the diet fed, and that a sheep which had been fed entirely on poor hay digested cellulose better than one not so adapted. To digest starch with maximal efficiency sheep need about a week of adaptation to a diet containing it. Van der Wath's comparisons of starch digestion were all done with such pre-adapted sheep. Just as Pearson & Smith(118) found no urease activity in bacteria-free filtrates of rumen liquid, so Van der Wath could find no diastatic activity therein. The digestion of starch in the rumen must therefore be entirely microbial, as both Baker and Van der Wath maintain. Van der Wath isolated an iodophilic streptococcus, responsible for starch digestion, from the rumen using Woodman & Stewart's medium(119). It did not produce gas when growing *in vitro*. The organism was obtained by suspending a silk bag, containing starch granules, in the rumen. The relative rates of digestion of various starches, already mentioned, were studied by this same technique. Baker & Harris(13) procured growth *in vitro* of a coccus which is responsible for cellulose digestion in the rumen. They used the medium which Vartiovaara & Roine(120) had used in studying digestion of wood pulps by pig, horse and steer. In these bacteriological studies of the breakdown of starch and cellulose in the rumen and elsewhere in the gut, all authors emphasize the importance of allowing the animal time to adapt itself to any change of diet. Diet changes the microbial facies in the ruminant, and it does in all other animals studied (Johansson & Sarles(17)). The change of microbial facies, or failure of the microbial facies to change fast enough, may have serious effects on the host animal's appetite or even on its health, and may well be a factor to be considered in certain forms of bloat or in ketosis. Thus Van der Wath found that readily distinguishable types of bacteria were collected in silk bags placed in the rumen of sheep according as the bags contained crushed maize, casein or cellulose. Evidently different bacteria preferentially attack different substrates in the one rumen at the same time, so that if the ratio of these three substrates in the diet is changed the facies will change also. Most interesting effects of adaptation of the sheep and its flora are described in the experiments of Hoflund, Quin & Clark(121), who measured the rumen's cellulolytic power by suspending cotton threads of definite length in it through the fistula. They found that cellulose digestion is best when there is 0·1–0·2% sugar in the rumen either more or less being inhibitory. The addition of sugar to hay stimulated both appetite and cellulose digestion. In each of two sheep fed 100 g. molasses, 20 g. casein and hay, cellulose digestion almost ceased when casein was omitted. When hay was eaten *ad lib.* and 50 g. casein per day were given through the fistula, a sudden increase of the casein to 100 g. per day reduced cellulose digestion almost to nil and also reduced the animal's appetite. Each of two sheep on poor quality hay were suddenly allowed access to lucerne leaves *ad lib.* and were given 100 g. per day of casein. These sheep gorged themselves with lucerne leaves and both became ill and cellulose digestion rapidly fell. One of the sheep died, and at autopsy was found to have a rumen smelling strongly of ammonia. Unfortunately blood ammonia was not determined so that it could not be definitely asserted that ammonia intoxication was the cause of death. Two more sheep were given lucerne hay leaves *ad lib.* together with 50 g. casein via the fistula. Intake rose rapidly and then declined in a few days to zero. Casein administration was then stopped and the animals gradually recovered both appetite and cellulose digestion. After recovery was complete 50 g. casein per day was given again but this time without mishap. Evidently the rumen flora of the animal had readapted themselves to the diet rich in protein. When sheep in these experiments were given a choice of hay or lucerne or a choice of hay or peanut meal, they ate a mixture of the two foods presented to them. Pounden & Hibbs(108) found that the type of ration fed to calves after the discontinuance of milk influenced the facies established in the rumen. The calves were all inoculated, after some time on the rations, by placing cud from full-grown animals in their mouths. The facies established varied in bacterial and protozoal composition depending upon the ratio of hay to grain in the calves' starters. Gall and her colleagues in a series of papers(113, 114, 115, 116, 117) have sampled rumen contents by stomach pump in the field, and have thus obtained comparative bacterial counts of dried smears from cattle and

sheep and also records of the facies present in them. They have also developed methods for culturing such samples anaerobically in sterile oxygen-free water. They found about 10^{11} organisms per g. rumen contents (115). Cattle and sheep fed similar rations presented a similar facies, and the pH was much the same in each species. When more grain was fed, more of the faster growing types of bacteria were present. Contrary to expectation, the feeding of silage did not have any marked effect on either the pH or the flora of the rumen. Many specimens of *Sarcina* were encountered in cattle and sheep at pasture, as also was the star-shaped organism reported by Baker (122). The latter organism was never seen in animals on winter rations. Cocci were more numerous when the rations contained more grain. All but two of the organisms isolated were obligate anaerobes, the two exceptions being facultative anaerobes. In other experiments Gall *et al.* (116) found less coccoid organisms adherent to plant fragments in the cobalt-deficient sheep.

Burroughs *et al.* (117) have studied the cultural properties of cellulose-digesting bacteria from the rumen of cattle, using filter-paper as a substrate. Cellulose digestion *in vitro* under conditions designed to simulate the microbiological picture obtaining in the rumen was accelerated by a complex salt solution containing trace elements, and also the constituents found by McDougall (90) in bovine saliva. It was also accelerated, though not quite so much, by ash or watery extract of dried alfalfa, by autoclaved rumen filtrate, or by autoclaved aqueous extract of cow manure. In these cultures daily transfers were done for several days.

McGaughey & Sellars (123) and Ingram & McGaughey (124) suggest that the organism in the rumen of sheep, *Schizosaccharomyces ovis* Quin, is not a schizomycete but a flagellate protozoan *Selenomonas ruminantium* (Certes, 1889). This organism stains brown but not blue with iodine. McNaught, Smith, Henry & Kon (125) have prepared dried rumen bacteria in sufficient quantity for their digestibility and biological value to be estimated. A comparison of their results with those published from other laboratories has been made by McNaught & Smith (14). Their bacterial samples are natural mixtures of what Baker calls 'the free iodophiles' of the rumen. McNaught *et al.* (125) found that the biological value of their bacterial preparation was $88{\cdot}3 \pm 0{\cdot}78$ (S.E. of mean), and the true digestibility $73{\cdot}2 \pm 0{\cdot}56$. The preparation contained 44·4% crude protein, 3·1% lipid, 0·3% crude fibre, 7·1% ash, 40·3% carbohydrate other than fibre. The small figure for crude fibre may be taken to denote a negligible contamination by plant debris. Calcium and phosphorus in this preparation were 0·29 and 1·35% respectively, and 4·4% of the carbohydrate was due to pentosans and other producers of furfural.

Further studies on the adaptation of rumen micro-organisms to variations of diet are being made by Bortree, Smith, Sarker & Huffmann (126), who reported the development in the rumen of an iodophilic 'great spirillum' when methionine was added to a ration of corn, starch, glucose and minerals. Adding grain or readily fermentable carbohydrate to hay caused rapid multiplication of rumen bacteria.

Marston (127), culturing rumen bacteria *in vitro*, found the main products of bacterial digestion of cellulose to be acetic and propionic acids, methane and carbon dioxide. There were smaller amounts of formic and butyric acids and traces of acetaldehyde, pyruvic and lactic acids. There were 1·5 parts of propionic to every one of acetic and 3–3·8 parts of CO_2 to every one of CH_4. Marston calculated that 85–88% of the combustible energy of cellulose was useful to the animal, provided the acetic was anabolized in some way. If this were not so, then only 67–72% of the energy of the digested cellulose would be available to the animal. In cold weather, of course, the whole of the deficit from 100% would help to spare the body energy stores by helping to maintain its temperature.

In a respiration calorimeter Swift, Bratzler, James, Tillman & Meek (128) have measured the methane production and energy metabolism of four sheep. Isocaloric rations containing 3–8% fat were used. It was found that methane production (grams) was related to carbohydrate (x g.) by the equation ($y = 2{\cdot}4x + 9{\cdot}8$), which differs from an earlier equation found for the cow ($y = 4{\cdot}0x + 17{\cdot}7$).

The function of roughage in the diet is a fascinating field for speculation, and involves paradoxes such as the occurrence of an optimum intake of cellulose in all animals tested for such an optimum. Thus even chicks do much better with a certain optimum (15) of cellulose in the diet

(Davis & Briggs(129)). It might be thought that its function is merely to form a substrate for intestinal synthesis of micronutrients forming the B-complex, but that this explanation may be too simple is shown by the last published paper of the late Sir Frederick Gowland Hopkins (Hopkins & Leader, see(2)) in which refection in rats was inhibited by allowing them to eat filter-paper. Axelsson(130) states that the optimum fibre content of the diet varies with species of animal considered, with the type of ration fed and with the external temperature. He (131) also maintains that heavy feeding for milk production necessitates food of higher protein (i.e. lower fibre) content. Blaxter & Price's(132) results confirm this observation. The effect of the rumen in facilitating fibre digestion is shown by Axelsson's(133) comparison of digestion in the pig and in cattle, sheep and goats. With the ruminants, protein digestion became negative when 35 g. protein were present in 1 kg. of dry matter. The pigs digested protein better, but they could not tolerate so great a concentration of fibre in the diet as could the cow.

In sheep and cattle digestibility fell as crude fibre was increased in the diet. The ruminants digested crude fibre better than did horses or pigs, as is shown in the following equations:

$$\begin{aligned} &\text{Cattle and sheep} && y = 87{\cdot}8 - 0{\cdot}83x, \\ &\text{Horses} && y = 88{\cdot}2 - 1{\cdot}25x, \\ &\text{Swine} && y = 93{\cdot}7 - 1{\cdot}60x, \end{aligned}$$

where y is the digestibility of organic matter and x is the percentage of crude fibre in the diet. The equations show that in the absence of crude fibre the pig has the best digestion, but that its digestion is most adversely affected by increase of crude fibre. From Vartiovaara & Roine's(120) figures this would appear to be true even of the pig with a very active caecal flora.

Axelsson(134) has made a statistical analysis of the metabolic trials with cattle in Denmark and Germany, with a view to relating maintenance requirements of cattle to body weight. For the German material he found the metabolizable energy to be $82{\cdot}7 \times (\text{body weight})^{0{\cdot}8}$ and for the Danish material $69{\cdot}1 \times (\text{body weight})^{0{\cdot}8}$. The power 0·8 was applicable to groups of adult animals, but for any one animal at different times a power nearer to unity was a better fit, while in experiments with uniovular cattle twins 0·85 was the best exponent. In experiments on rabbits, Axelsson(134) found that metabolizable energy was independent of the content of dry matter in the rations. Blaxter & Price(132) showed that Britain's heifers did not take well the increased fibre content of wartime rations as judged by growth rates. On such a ration extra protein but not extra starch increased growth rates.

Breirem(135) states that the feeding of cellulose to cows increases the fat in the milk at the expense of the milk yield. The same author has calculated that grass converted into milk by ewes yields 25–30% less energy than would be obtained if the lambs consumed the grass direct. Burroughs & Gerlaugh(136) and Burroughs, Gerlaugh, Edgington & Bethke(137) added soyabean to the rations of steers to increase the protein from 8 to 15%, and found that the digestibility of the dry matter of the ration which contained corn-cobs and timothy hay was greatly enhanced. These experiments recall the negative protein value of fodder-cellulose quoted by Vartiovaara & Roine(120). In later experiments the same authors found that protein as skim milk helped digestion of corn-cobs, especially when added to the diet with starch. Starch alone did not appear to have such an effect. Later still, the same authors(138) confirmed the finding that roughage digestion in a ration of alfalfa hay and corn-cobs was depressed by successive additions of starch from 63 to 32%. Addition of skim milk raised this digestibility to 48%. Burroughs, Gerlaugh & Bethke(139) had earlier shown that the digestion by cattle of the roughage in corn-cobs was much better when alfalfa ash or watery extract of alfalfa was added to it.

Some interesting botanical observations on the selective habits of grazing sheep have been made by Cook, Harris & Stoddart(140). Sheep on range are shown to be highly selective and to tend to choose only leaves and the tenderer stems.

Gallup & Briggs(141) found that steers obtained total digestible nutrients increasing from 41 to 56% as the quality of their diet of hay increased from 3 to 6% protein. At the same time the digestibility of crude fibre increased from 56 to 69% and that of the protein from negative values to 41%. When cottonseed meal (0·5–3 lb. per day) was used to supplement 10 lb. of hay,

differences of digestibility were again apparent, for the digestibility of the dry matter of the good quality hay was equalled by that of poor quality hay plus 2 lb. cottonseed meal. Faecal nitrogen was 0·559 g./100 g. dry-matter intake from hay (of any protein content) and 0·779 from hay supplemented with cottonseed. Forbes(142) has demonstrated by further experiments that, contrary to a suggestion by Gallup & Briggs, faecal nitrogen output cannot be used as a measure of the dry-matter intake of grazing animals. He has shown that on fresh grass faecal nitrogen varies but not in a predictable manner.

Blaxter & Mitchell(143, 144) suggest that changes of metabolic faecal nitrogen preclude the making of estimates of protein requirements of cattle without reference to the character of the ration. Louw, Bodenstein & Quin(145) kept sixteen sheep on poor veldt hay containing 2·2% protein for 41 days, during which time there was a gradual decrease in the degree to which they digested cellulose and they all lost weight steadily. Varying amounts (20, 50, 85 and 170 g.) of a supplementary mixture of starch 76 parts, casein 15 parts, yeast 5 parts, and mixed minerals 4 parts were then given to each of four sheep. Loss of weight was reduced accordingly, but only the group of sheep getting 170 g. started to gain weight. A fall in cellulose digestibility was associated with the 170 g. supplement, but not with the others. When all the sheep were given 200 g. green lucerne, cellulose digestion improved in all the animals, but the improvement was out of all proportion to the small amount of extra young cellulose provided by the supplement.

Harshbarger & Salisbury(146) found the greatest gains in weight, and the expenditure of total digestible nutrients per lb. of live weight gained, were given by rations containing 70% roughage. Rations with either 55 or 94% roughage were inferior when judged by these two criteria.

Steensberg(147) has, from experiments done by himself and predecessors in Denmark, made a very enlightening examination of the requirements of three different breeds of Danish cattle, from 16 days of age until after the first calving at 27 months. Several hundred head of cattle were studied. Considerable variations in the level of feeding are reflected by pronounced differences in growth, body size and live weight. Between the three breeds, Red Danish Milk, Black and White Jutland and Dairy Shorthorn there were only slight differences in growth. The effect of a low intake of food in the winter is largely counterbalanced by good pasture in the summer, since after 9 months of age the animals can subsist entirely on pasture in the summer. Differences in live weight and development at first calving, resulting from differences of plane of nutrition during the 27 months, tended to disappear in the course of the first two lactation periods when all the cows were fed alike and according to accepted Danish standards. A considerable part of the leeway was made up while the cows which had been underfed were on pasture. Feed consumption per kg. in weight increases greatly with increasing age, and this is true whether the total consumption of starch equivalent or merely that portion contributing to growth (the so-called production S.E.) is considered. Total S.E. required to produce 1 kg. gain in weight is much the same at the same age for the heifers in the high, medium or low plane of nutrition. This, says Steensberg, is because the proportionately larger maintenance requirement of a smaller animal just counterbalances the proportionately smaller production requirement of the smaller animal. For these heifers, 300 g. digestible pure protein was insufficient for normal growth, for which 375–400 g. was necessary for animals above 24 months of age. At that age 3·5 kg. starch equivalent per day are needed. A table of S.E. and protein requirement of female cattle beasts of various ages is given by Steensberg.

From respirometer studies on seven merino ewes at five levels of food intake from $\frac{1}{2}$ to $2x$ maintenance, Marston(148) found that after 72 hr. fast sheep previously at twice or half maintenance were evolving 74·5 or 59 cal./$W^{0.73}$/day respectively. In the former state the animals were not post-absorptive, while in the latter there was inanition. He then from animals at all levels calculated 68 cal./$W^{0.73}$/day as the ewe's fasting metabolism.

Musgrave & Gaines(149), comparing milk yields of cows at various food intakes, have concluded that the effect of the cow's initial weight on milk yield is greatest at lowest intensities of feeding. Robinson(150) has made a study of the effects of partial starvation and realimentation on ewes by analysis of carcasses slaughtered at predetermined dates. Rate of loss or gain of muscle was linear, but bone weight did not change much. Initial changes of kidney and liver were rapid and

were followed by more gradual ones. Increase of weight on realimentation was much faster than loss of weight resulting from deprivation of food.

The relative efficiencies of animals in converting plants into human food is of great importance to Britain and the reader is referred to the detailed presentations on this topic by Wright(7), Godden(6), Maynard(151) and Leitch & Godden(152).

Huffman & Duncan(153), by replacing a portion of the all alfalfa hay ration of twelve cows by its equivalent of total digestible nutrients in corn, found a marked increase in the production of milk (corrected to 4% fat). When the cows were again replaced on the all hay ration a precipitate drop of milk yield occurred. These changes are attributed to some specific factor or factors in the corn. The milk yield responded very rapidly, within a day, to the ration changes and corn was far more effective in producing the change than were corn-starch, corn-sugar, soyabeans, cane molasses or sugar. The authors note the similarity of their result to that of Davis & Kemmerer(154) who posited specific lactation factors in grapefruit peel, and of Smith, Jones & Haag(155).

When alfalfa hay was supplemented by whole soyabean meal the milk yield of cows was no different from that obtained when defatted soyabean meal was eaten. Nevertheless, the extra oil in the former ration gave rise to milk with a greater percentage of fat. Leroy & Bonnet(156) found that cows show a reduction of both fat yield and milk yield if there is less than 3% fat in the feed, and that a cow should eat 350 g. fat per day. On normal rations fat content is, according to these authors, negatively correlated with milk yield. If, however, fat-deficient rations are fed this correlation becomes positive. High yielders, they say, respond to a cut in fat by a lowered yield, low yielders by a reduction of percentage of fat in the milk.

Lignin is almost unattacked on passing through the gut even of the ruminant, so that it has been used as a natural marker in determining digestibilities in the animal(157, 158, 159). An agreed method is needed for determining lignin, the more particularly as it is not a definite chemical entity(160, 161, 162), and the lignin or any added marker such as ferric oxide(163, 164, 165), or chromium sesquioxide(166, 167, 168, 169), must be intimately mixed with whatever constituent of the ration is having its digestibility determined. The disadvantage of a natural marker such as lignin, silica (164, 170), or a natural pigment(171), is that it cannot be so mixed. The advantages of chromium sesquioxide are its physiological inertness, its fineness (which militates against gravitational separation in the rumen or elsewhere in the gut) and its virtual absence from any ration, or part of a ration, unless it has been added deliberately. Constituent silica proved useless with silage(170). There is a tendency for a gravitational separation, in the rumen, of constituents, as indeed is obvious from post-mortem examinations, so that one cannot rely on mastication and rumination to mix thoroughly foods not already mixed before being eaten. Marshall(158), using the lignin method, found that 45% of the pentosans in hay were digested by two sheep. More than half this digestion occurred in the rumen and omasum, and the remainder in the caecum and colon. Pazur & DeLong(172, 173), as part of a series of studies of the chemistry of pastures, have measured the digestibility of lignin itself by measuring excretion of aromatic acids. Clover cut at five stages of growth was fed to sheep. The urinary excretion of hippuric and benzoic acids was three times as great with young as with mature clover. The authors suggested that the difference was due to different susceptibilities to bacterial attack, but no bacteriological data were produced. A direct determination of the digestibility of lignin in young and old forages is much to be desired.

Gray(157) fed sheep a constant diet of lucerne hay and wheat straw for several weeks before slaughter and then, by examining post-mortem the ratios of cellulose to lignin in various parts of the gut, deduced that, of the digestible cellulose in the food, 70% was digested in the rumen, 17% in the caecum and 13% in the colon. In each of these sites bacteria abound and lower fatty acids pass from the gut to the blood.

Maymone & Tiberio(174) used four different methods for estimating crude fibre in food and faeces of sheep. The crude fibre in food and faeces varies so much with the method that the digestibilities were widely divergent. Similar observations are reported by Nordfeldt, Svanberg & Claesson(175).

Pazur & DeLong(172, 173), by measuring hippuric and benzoic acid excretions of sheep, deduced

that lignin in young plants is more metabolized than lignin in older plants. The bearing of this finding on the use of lignin as a marker is not discussed by the authors.

Drapala, Raymond & Crampton(176) and Norman(177) have correlated histological studies of red clovers with their digestibility in sheep, and given added support to the generally held belief that older plants are less digestible than younger plants, because of their greater degree of lignification. Photomicrographs of lignification of faecal residues were prepared.

Fournier(178) and Huisman(179) have studied effect of cell membrane in food on digestibility. Hvidsten(180) found that the digestibility of spruce sulphate pulp was related to its lignin content by the equation $D = 84{\cdot}9 - 1{\cdot}15x$, where D = digestibility and x = percentage lignin in the preparation. Druce & Willcox(181) have elaborated a new rapid method of estimating 'cellulose' obtained from hay, grass, alfalfa, wheat bran and faeces of the pig and the cow. In another paper these same two authors have applied their method to determine the digestibility by two rabbits of the fibre of a diet of oats, maize, bran, fish meal and dried grass. Digestibilities of cellulose by the Norman & Jenkins method were 23·7 and 29·3% respectively, while the corresponding figures for Druce & Willcox's method were 29·8 and 28% respectively. The corresponding figures for Crampton & Maynard's(182) method were only 11·9 and 11·0% respectively. It is concluded that Crampton & Maynard's method isolated an abnormally large proportion of putative cellulose from rabbit faeces.

Naumann(183) and others(174, 175, 184) provide evidence for the very general belief that the so-called 'crude fibre' of plants is a very mixed bag. Naumann has shown that samples of crude fibre prepared by the Weende method from a variety of sources have different chemical properties, but that the samples of crude fibre prepared from the faeces of sheep to which these sources are fed are much alike in composition. Digestibility of the fibres was inversely proportional to the lignin content which, in most cases, was indigestible. Cotton-wool, which is almost pure cellulose, was 90% digested by the sheep. It is concluded that assessment of the value of a diet is much limited by variability of the composition of crude fibre.

Davis, Miller & Lindahl(185) observed digestibilities in ewes of 10·6 and 16·2% for the lignin of pea and lima bean vines respectively.

Jacquot(184) suggests that the material insoluble in formic acid be used as an index of the indigestible matter of feeding stuffs. Reid *et al.*(171) have hit upon a natural variant of the method of determining digestibilities by means of dyed straw. They found that a natural chromogen present in grass and absorbing at 406 mμ. was 100·5% recoverable in thirty-six trials. The chromogen would, they say, be useful in checking results from lignin ratios because grass which is refused is rich in lignin and low in chromogen. Snowden & DeLong(186), after studying lignin fractions from pastures and from the faeces of ruminants, have concluded that lignin, because it is not a definite chemical entity and because it varies with plant maturity and species, is not suitable for use as a marker in experiments on digestibility. Swift, Thacker, Black, Bratzler & James(159) found by comparing lignin ratios with direct measures that of nine pairs of figures only four showed significant differences. They were comparing the effect of various supplements at two levels on the digestibility of a diet by wether lambs. The basal diet contained alfalfa hay, timothy hay, maize meal and linseed cake meal. The supplements were oat straw as a source of crude fibre, maize sugar and starch as carbohydrate and casein, or urea or maize oil. When straw was added to the basal diet digestibilities of protein, ether extract, crude fibre and energy were reduced. Both types of carbohydrate reduced the total amount of crude fibre and crude protein digested. Casein, and to a less extent urea, reduced the digestibility of crude fibre. Maize oil improved digestibility of all constituents at lower and made them worse at higher levels of supplementation. Watson, Kennedy, Davidson, Robinson & Muir(187) measured the digestibility by cattle of rations of nutritive ratios varying from 1:2 to 1:9. No effect of the nutritive ratio on the digestibility was observed. Watson, Davidson, Kennedy, Robinson & Muir(188), by adding barley to hay so as to produce rations of three-quarters to one and a half times the estimated value for maintenance of six grade Shorthorn steers, found that as the plane of nutrition increased the digestibility of the barley decreased. The same authors(189) found no difference in the powers of sheep and steer to digest a wide variety of animal feeding-stuffs.

Hvidsten & Homb(190) in reporting digestion trials with spruce wood, cellulose and alkali-treated straw stated that, in 1940–44, three-quarter million tons of cellulose were used as fodder in Norway.

Hvidsten(180) found that 37 g. extra protein had to be fed to cattle to enable them to digest 1 kg. cellulose. Feeding of cellulose lowered the yield of milk but increased its fat percentage.

Forbes & Garrigus(191), after feeding various forages to steers and wethers, found that the amount of lignin in the faeces was apparently 1–23% (average 14%) greater than the amount in the feed.

EFFECT OF DIET ON THE VIABILITY AND THE BIRTH WEIGHT OF YOUNG

Williams, Sylvestre, Bowstead, Ewen, Myhr & Peters(192) found greater birth weight and vigour in lambs from ewes receiving legume hay than in those from ewes on grass hay. The superiority of the legume was not due to vitamin A. Similar effects of diet on birth weight and development were found by Thomson & Thomson(105). Hansson(193) has studied the weight changes of 124 cattle during pregnancy, and has found little change of weight during the first 3 or 4 months. From the end of the 4th month till calving, weight increased, especially in lactating animals. Loss of weight on calving was 57·3 kg, of which 62·3% was the weight of the calf. Average birth weights of calves were 32·9, 35·5 and 37·0–37·6 kg. for the 1st, 2nd and 3rd or higher parturitions respectively. Birth weight rose with the live weight of the cows and most of the live weight increase of the cows was foetal. These data have an obvious bearing on experiments designed to show the effect of prenatal feeding on birth weight. Putnam & Henderson(194) have made a similar study.

Wallace(195) has made an extensive study of lamb production by sheep. By weighing lambs he found that high-yielding ewes during the 1st month required little more than ½ lb. gross digestible energy for each lb. of milk produced, while low yielders during late lactation might need 121 lb./lb. milk. Shapes of the lactation curves for ewes were also found by weighing lambs before and after suckling over a 24 hr. period once a week. Over a 16-week lactation the following percentages of milk were produced: 38, 20, 21 and 11 in the 1st, 2nd, 3rd and last month respectively. These studies also illustrate the importance of good pre-natal feeding of the ewe on the birth weight and adequate suckling of its lambs. It is shown that twin lambs give a better index of potential milk production than single ones. A single lamb does not draw off all the milk the ewe is capable of producing.

Quinlan, Roux, van Aswegen & de Lange(196) showed that restriction of activity and access to sunshine did not affect the reproductive performances of South African beef heifers and cows. They observed that older cows had on the average lighter calves, and one wonders whether this was a nutritional effect. Hart, Guilbert, Wagnon & Goss(197) report a congenital deformity called 'acorn calves' which is induced by faulty nutrition of the dam.

DIET AND THE BULL

Larsen & Sørensen(198) found that the bull's diet did not influence the volume of ejaculate, but that increase of protein intake markedly increased the number of living sperms per μl. of semen.

As judged by rates of growth, blood composition and rates of semen production, the feeding of bulls on timothy-clover hay plus a complex concentrate was in no way superior to using a simple, much less expensive, concentrate mainly of corn and its by-products (Reid, Ward & Salsbury(199)).

NUTRITION OF CALVES

Esh & Sutton(200) found that in the newborn calf, as in other animals, nicorinic acid can arise from tryptophan, and that *N*-methyl nicotinamide and other nicotinic acid derivatives are among the forms in which it is excreted. Krupski, Almasy & Ulrich(201) report that the trouble that results when calves are too quickly changed from milk to an ordinary ration is chiefly due to the

negative balance of calcium which results. Warner & Sutton(202) destroyed 96% of the riboflavin and 75% of the vitamin A in milk by irradiation with a mercury vapour lamp. Such milk with added A produced ariboflavinosis curable by extra riboflavin, in calves to which it was fed. Sutton & Esh(203) found that the first milking contained five times, and the second three times the tryptophane content of later milk, thus explaining the earlier observations of Johnson, Wiese, Mitchell & Nevens(204) of initial large excretions of nicotinic acid by calves in their experiments with nicotinic acid. Nevens & Kendall(205) could not find much benefit from supplements of vitamins A and D to calves during the first 30 days of life. Nicotinic and ascorbic acids had no effect at all. Incidence of scours was one of the criteria used. Arrington & Reaves (206) reported that Holstein calves thrived on reconstituted skim milk on which Jerseys failed to survive.

Murley, Jacobson, Wise & Allen(207) found comparable growth with either butterfat or soyabean oil, but soyabean oil whether crude, refined or hydrogenated, caused more scouring than butterfat did. Barker & Jacobson(208) found that there was little variation of fat in blood plasma, whether crude or hydrogenated soyabean oil or butter were used as fillers for reconstituted milk.

McCarthy & McDougall(209) immunized ewes against *Bacterium typhosum* H in the second half of pregnancy, and found that antibodies in the colostrum could be absorbed by lambs up to 29 hr. after birth but not after 48 hr. or more. During the 1st day after birth lambs showed a marked but transient passing in the urine of protein containing the immune globulin. Occasional lambs which had not suckled showed antibody in the blood but in amounts which were negligible compared with amounts found in lambs which had suckled. Parrish, Wise, Hughes & Atkeson(210) found that extra protein pre- and post-partum did not affect the protein content or casein number of the milk of ten cows as compared with ten unsupplemented controls. The mechanism of formation and transfer of immunity by colostrum has been briefly reviewed by Blakemore(211) and Kerr(212). Hansen & Phillips(213) found that the reason why calves more than 2 days old do not benefit from colostral antibodies is in part due to the greater extent to which they digest the antibodies, and in part to changed permeability of the gut with age. Aschaffenberg, Bartlett, Kon, Walker, Briggs, Cotchin & Lovell(214) found evidence of a protective factor on the watery phase of milk.

Marshall, Becker & Davis(215) found shark meal an effective substitute for skim milk in calf rearing. Wiese & Johnson(216) found that calves given liberal amounts of vitamin A did not require vitamin C. They also found lard to cause less scouring than did soyabean oil. Erb, Scott, Norton & Morrow(217) found that supplements at birth of vitamins A and D and nicotinamide did not benefit the health or well-being of calves during the first 2 months of life. Incidence of scouring was correlated with quality of the barns in which the animals were housed.

Jarvis & Waugh(218) and Jacobson, Cannon & Thomas(219) have demonstrated the beneficial effect of hydrogenation of cottonseed and soyabean oils when used for feeding with milk substitutes to calves.

OCCURRENCE OF OESTROGEN IN FORAGE PLANTS

Though the occurrence of oestrogens in plants is no new observation, their sudden appearance in toxic quantities in subterranean clover in parts of Western Australia (Bennets(220)) has stimulated speculation as to the significance of non-toxic amounts naturally present in animal feeds. No attempt will be made to review the growing literature on the use of stilboestrol to increase meat production by farm animals. Neither will any attempt be made to review the use of stilboestrol for artificially inducing and for stimulating or inhibiting lactation, except to mention that one of the virtues of stilboestrol is its activity when given by mouth. Bennetts has made a more detailed study of the anatomical changes in the sex organs in forty-five wethers grazing potent green clover. He has also shown that similar changes are caused in wethers by stilboestrol, and that male sheep which have not been castrated are unaffected by grazing subterranean clover. The effect of the clover on pregnant ewes is, like that of stilboestrol, to cause abortion. East, Underwood & Bennetts(221) have demonstrated that, in the castrate male sheep, androgen is protective against the harmful effects of subterranean clover, thus explaining the immunity of the intact male from subterranean clover disease in the field. Legg, Curnow & Simpson(222) have

studied the seasonal distribution of oestrogens in two species of rye grass, in cocksfoot and in red clover. Their preliminary figures are consistent with the hypothesis of Bartlett, Folley, Rowland, Curnow & Simpson (223) that a somewhat greater than usual, but still non-toxic, concentration of oestrogens in spring as compared with autumn grass leaves may be responsible for the superiority of spring grass as a milk producer. Schinckel (224) has reported the occurrence of infertility of ewes grazing subterranean clover in Kangaroo Island, South Australia. Robinson (225) has described the assay of subterranean clover extracts for oestrogenic potency.

VITAMIN A AND CAROTENE

Recent work (226, 227, 228, 229, 230, 231, 232, 233, 234) shows that the site of conversion of carotene to vitamin A in rat, pig and goat is the wall of the small intestine and not the liver. The vitamin A resulting from this conversion is absorbed chiefly by the lymph and thence passes to the blood and ultimately to the liver where it is stored as vitamin A ester. Carotenes are also absorbed, in animals with yellow milk, unchanged and stored in the liver but it is not known whether such carotenes have a physiological function. The paradoxical result has been observed by Sexton, Mehl & Deuel (235) of rats suffering from vitamin A deficiency with an apparent abundance of carotene in their livers. The carotene was injected into these animals so that the wall of the gut was by-passed. Liver stores of carotene in experimental animals thus resemble those of cobalt (236) in not necessarily reflecting the animal's nutritional status, though they probably are a reliable index in the field.

Goodwin & Gregory (232) could not demonstrate carotene in either portal or systemic blood of rabbits, goats or sheep after massive doses of carotene. This was true not only of oral doses but also of doses given by abomasal or duodenal fistula. Carotene as dispersed red palm oil was as good a source of vitamin A as homogenized spinach. After dosing with vitamin A there was much more in intestinal lymph than in the blood plasma. In spite of this, lymph in goats is usually less rich in vitamin A than is blood plasma. The surprising result is reported by Goodwin and Gregory that goats have no carotene in adrenals, liver, ovaries or bone marrow. Goats, however, vary in this respect, for there is appreciable carotene in the colostrum of the goat, and of four goats examined by Chanda & Owen (237) two had β-carotene in the liver while another had only a slight trace, while the remaining one had none.

Eden & Sellers (238) studied the absorption of vitamin A by bullocks, sheep and rats, some dosed before slaughter and others not. In animals receiving vitamin A but not carotene there was a greater rise in the systemic than in the portal blood plasma. In bullocks, it was shown that lymph from the intestine was richer in vitamin A than that of any other part of the tract except the duodenum. Elliott (239) found decreasing concentrations of carotene in the veins as he proceeded along the intestine. He also noted the inability of the calf to convert transfused carotene into vitamin A. These facts are consistent with the hypothesis of the intestinal conversion of carotene to vitamin A. Evidence is still accumulating that the thyroid affects the metabolism of vitamin A (Wiese, Mehl & Deuel (240)), and the translocation of hepatic vitamin A within the body (Johnson & Baumann (241, 242); Heimer, Marlow & Sobel (243)). Eveleth, Bolin & Goldsby (244) found that, in sheep, the increase of vitamin A in blood plasma after carotene ingestion was much reduced if the goitrogen thiourea was given with it. Cama & Goodwin (245), using rabbits, found that thyroxine acts on the metabolism of vitamin A by facilitating the intestinal absorption of carotene, but has no effect on the plasma vitamin A levels. Cama & Goodwin used the goitrogen thiouracil in control experiments in support of these findings.

Meunier, Ferrando, Jouanneteau & Thomas (246) found that vitamin A facilitates the formation *in vivo* of hippuric acid from benzoic acid, and concluded that vitamin A is essential for the synthesis of glycine. Winzenried & Wanntorp (247), by comparing uniovular twins, concluded that vitamin A, total carotenoids and carotene in the milk and the extent to which the organism can convert carotene to vitamin A are a part of the cow's inheritance.

Whether the protective action of colostrum when fed the calf is due to its richness in vitamin A or in antibodies against 'white scours' is still in dispute. Blakemore *et al.* (211) showed that the

protective action of colostrum and precolostrum is associated with its globulin content. Aschaffenberg *et al.*(214), by feeding different fractions of colostrum to calves, correlated health with the watery phase rather than the fatty phase of colostrum. Both these groups therefore minimize the importance of the vitamin A content of colostrum.

Pope, Phillips & Bohstedt(248) compared the blood chemistry of ewes and their lambs. Corresponding with the richness of colostrum in vitamin A there was a sharp rise in the first 2 days in the plasma vitamin A of the neonatal lamb. The rise of plasma vitamin C in the lamb's blood was also sudden but was delayed. This is presumably because colostrum is not rich in vitamin C and because the lamb's synthetic powers take a few days to develop.

Parrish, Wise, Atkeson & Hughes(249) found that access to pasture increased the carotenoid content of colostrum and transitional milk, but had only a small effect on its vitamin A content. Doses of one-half or one million i.u. vitamin A increased vitamin A but not carotenoids in colostrum and transitional milk. One and a quarter million i.u. lowered the carotenoids. There was always about three times as much vitamin A in the first colostrum as in milk a fortnight later irrespective of supplementation with vitamin A. Parrish, Wise & Hughes(250) found that in 4 weeks' pre-partum supplementation these was no evidence of a superiority of either the alcohol or ester of vitamin A ($\frac{1}{2}$–1 million i.u.) and that the supplementary effect was not enhanced by 10 g. mixed tocopherols per day. Fountain, Parrish, Atkeson & Hughes(251) noted that the vitamin A and carotenoids in the blood of the newborn calf were greater from dams at pasture than from dams fed on grain. Savage & Heller(252) noted a direct but delayed relationship between blood carotene and intake of grass. Stewart & Watts(253) noted a similar correlation of liver carotene and vitamin A with intake of grass throughout the year. Krijt(254) found that pasture maintained vitamin A in goat's milk better than did dried grass or hay. Marq & Rouir(255) made similar observations. Wiseman & Shepherd(256) found vitamin A in butter to be greater after silage than after hay, but the vitamin D activities were not compared. Changes in the carotene content of the ration caused by using oat straw instead of alfalfa were found by Weir, Pope, Phillips & Bohstedt(257) to be reflected in the vitamin A of colostrum, blood plasma and liver of ewes and of the liver of their lambs. Twin lambs of ewes given straw grew slower than single ones, and extra vitamin A did not enable them to catch up. Eden(258) showed that the rapid drop of vitamin A in the ewe's colostrum from 1830 to 130 i.u.% in 72 hr. took place while the vitamin A in the lamb's plasma rose from 20 to 90 i.u.% in 12 hr. and then fell back to 20 over the next 48 hr. Spielman, Eaton, Loosli & Turk(259) found that pre-partum supplements of alfalfa meal, vitamin A ester or vitamin A alcohol had little effect on the vitamin A and carotene in the blood stream of their calves. Parrish, Wise & Hughes(260) found that the increase of plasma A in pre-partum supplemented cows was in ester form, while in newborn calves it was mostly alcohol in the plasma and ester in the liver. Carotene formed about 97% of the carotenoids in cow's blood and 94% in calf's blood. Anatharamiah, Anantakrishnan & Sen(261) found that a ninefold increase of carotene intake trebled the vitamin A potency of the fat of buffalo's milk. Parrish, Wise & Hughes(262) supplemented cows with vitamin A and found that either the alcohol or ester form of it increased the reserves in the liver of the newborn calf.

Ross, Knodt & Guerrant(263) showed that within 10 days of supplementation with β-carotene, vitamin A alcohol or natural vitamin A esters, growth was resumed by vitamin A-deficient heifers maintained on a ration of oat straw and concentrates. With both forms of vitamin A supplementation plasma-A increased but with β-carotene plasma-A decreased. After withdrawal of supplements growth again ceased when the vitamin A in the plasma fell to the critical level of 6–8 mg./100 ml. Wise, Caldwell, Parrish, Atkeson & Hughes(264) studied vitamin A in the calf's blood from birth to 10 weeks of age, and found the variations to reflect the intake. Thus after an initial sharp rise, due to colostrum, there was a gradual fall until hay which was available from the 14th day was being eaten in appreciable amounts. A similar but longer investigation with sheep had been earlier reported by Pierce(265). Eaton, Spielman, Johnson & Matterson(266) found less vitamin A and carotene in the blood plasma of calves whose dams had been milked pre-partum, than in the plasma of normal dams' calves. Elliott(267) found 3·2 mg. carotene per day per 100 lb. live weight to be insufficient for normal growth or to maintain adequate plasma levels or liver

stores. Requirements were 6 mg. for Holsteins and 10 mg. for Guernseys. Moore, Sykes, Jacobson & Wiseman (268) found 3·4–3·6 mg. from alfalfa meal sufficient, but 2·8–3·2 insufficient to prevent a rise of cerebrospinal fluid pressure. For Holsteins and Ayrshires 3·0 mg. was the necessary minimum carotene per day per 100 lb. and, say the authors, this is significantly lower than Guernseys' requirements.

Jacobson, Converse & Moore (269), in experiments on fifty-two calves, found that depletion of stores till vitamin A in plasma was less than 4 mg./100 ml. took 4 months if the calves had been 90 days at pasture. Bratton, Salisbury, Tanabe, Branton, Mercier & Loosli (270) and Madsen, Eaton, Heemstra, Davis, Cabell & Knapp (271) have studied the effects of vitamin A deficiency in bulls. In addition to the classical symptoms they observed an increased percentage of abnormal spermatozoa and a decrease of motility of spermatozoa, together with histological changes in the testes. The testicular condition was curable by vitamin A, but was unaffected by injection of vitamin C. Esh, Sutton, Hibbs & Krause (272) reported that soya lecithin increased vitamin A in the colostrum of twenty-six dairy cows. Vitamin A itself seemed to lower the carotene in the colostrum. By contrast Eaton, Christian, Daugherty, Spielman & Matterson (273) found that the increase in vitamin A in blood of ewes or sows, or their offspring, or in the vitamin A of the livers of their offspring, were unaffected by simultaneous ingestion by the dams of soya lecithin. Squibb, Cannon & Allen (274) found that raw soyabeans reduced the blood plasma carotene of cows, but that the reduction did not occur when soyameal free from oil was used. Ellmore & Shaw (275) found that raw soyabeans reduced vitamin A in plasma also. Aqueous dispersion of vitamin A and of carotene in oil prepared by the use of surface-active substances facilitates absorption (Sobel, Sherman, Lichtblau, Snow & Kramer (276); Wise, Jacobson, Allen & Yang (277)).

VITAMIN D

Roughages, being the only animal food commonly subjected to insolation after harvest, are the chief source of vitamin D in cattle and sheep rations. Moore, Thomas, Jacobson, Melin & Shepherd (278) record that the antirachitic value of alfalfa hay is 1·5 to 2 times as great when it is cured in the field as when it is barn-dried or wilted and made into silage. Tests on calves kept in the dark from birth to 8 months of age nevertheless showed these increases to be unnecessary. At all stages of growth of the alfalfa the final content of vitamin D was greatly affected by the great concentration (3200 i.u. per lb. of dry matter) in the dead leaves adherent to the plant when harvested. No vitamin D was detectable in live green leaves. Similar determinations have been made by Newlander (279). Wallis, Smith & Fishman (280) found that oat and vetch made the poorest and peanut the richest source of sun-cured hay. Alfalfa hay was intermediate. Dehydrated alfalfa meals were better sources than alfalfa silages. Spitzer, Marayama, Michaud & Phillips (281) found that the effect of vitamin D in increasing the utilization of phytic phosphorus could not be attributed to any effect on the activity of phytase. In experiments with rats Norman & Mittler (282) have shown that the female sex hormone is antirachitic while the male sex hormone is rachitogenic. Both hormones stunted growth. Leinzinger (283) could not demonstrate any oestrogenic activity of vitamin D. Nordfeldt (284), using P^{32}, has shown that one of the actions of vitamin D is to favour the metabolism of bone at the expense of the soft tissues, while Migicovsky & Emslie (285), using Ca^{45}, demonstrated the same effect. This is the exact opposite of the effect of thyroxine (Owen (286)).

VITAMIN E (TOCOPHEROLS)

Probably, because of the extreme difficulty of keeping ruminants healthy for any considerable time on rations free from natural roughage, the function of tocopherols in ruminant metabolism is still puzzling and still presents apparently contradictory findings. By analogy with animals so diverse as the guinea-pig, the rabbit and the chicken, it is difficult to deny that the toxicity of cod-liver oil in moderate doses is due to its induction in these animals of a vitamin E deficiency. With massive doses the toxicity of excesses of vitamin A and of vitamin D may also supervene. A certain fraction of the unsaturated fatty acids seem to catalyse the oxidation of vitamin E

in vivo. These same acids also account, according to Hilditch, for the fact that cod-liver but not shark-liver oil (both of these oils contain both vitamins A and D, and can therefore increase the vitamin A and D potency of butterfat) depresses the yield of fat in cow's milk. Recently, Ferrando, Chenavier & Cormier(287) have found that the depression of butterfat percentage in the milk of the cow by cod-liver oil is accompanied by a fall of the level of tocopherol in the blood, and that these effects can be reversed by giving maize germ oil as a source of vitamin E. Cormier(288), in a review of the antagonism between cod-liver oil and vitamin E, concluded that the specific factor or factors causing the toxicity of the former is not yet accurately characterized. Meunier, Ferrando & Chenavier(289) recorded that young sheep given maize germ oil were adversely affected by dosing with cod-liver oil. They stopped growing, became less active and exhibited a fall of blood tocopherol to less than half. Much of the work on the function of tocopherols in ruminants is incapable of interpretation, due to lack of comparable control animals (Gullickson, Palmer, Boyd, Nelson, Olson, Calverley & Boyer(290); Cotchin(291), Marsh(292)). Gullickson, Fitch & Gilmore(293) could see no effect on the milk yield, milk composition or wellbeing of eight cows receiving 1 g. per day of mixed tocopherols in addition to an ordinary balanced diet as compared with controls. Krukovsky, Loosli & Whiting(294) gave cod-liver oil drenches to one of a pair of cows. The iodine value of the milk fat, as would be expected from the work of Hilditch and his school, increased. The tendency of the milk to produce an oxidized flavour on standing was correlated directly with the iodine value and inversely with the tocopherol content of the butterfat.

Whiting, Loosli, Krukovsky & Turk(295) found that 5 oz. of cod-liver oil decreased the fat production by 14% and increased the milk production by 6% in sixteen cows. Each cow received each of four diets, one after the other. One diet had extra vitamin E added, another had cod-liver oil. A third diet had neither addition, and a fourth both. Tocopherol did not counteract the effect of cod-liver oil on the milk. The tocopherol content of the milk was increased by giving tocopherol and reduced by cod-liver oil. Cod-liver oil had a depressant effect on the tocopherol content of both milk and blood plasma, but these effects were counteracted by feeding tocopherols. The vitamin A levels in milk and in blood plasma were increased by cod-liver oil.

In experiments of Phillips, Kastelic & Hart(296) 1 g. mixed tocopherols per head per day had no effect on the fat percentage or yield of milk or on the amount of tocopherol in the blood plasma. In calves, 10 days to 2 months old, muscular dystrophy ('white muscle disease') may appear spontaneously (Vawter & Records(297)). Parrish, Wise & Hughes(298) have shown that colostrum from cows contains about 100 mg. vitamin E per g. fat which declines to about 20 mg. on the 16th milking. When 0·5 g. vitamin E was given daily pre-partum the colostral but not the milk figure was increased, while 10 g. vitamin E per day increased the colostral value by five times and doubled the value of the 16th day's milk. Similar observations were reported for ewes, goats and sows by Whiting & Loosli(299) and by Latschar, Wise, Parrish & Hughes(300). Tocopherol was found by Whiting, Loosli & Willman(301) to facilitate the placental transfer of vitamin A to the foetal lamb but not to the kid or the piglet. Parrish, Wise & Hughes(302) were unable to find any effect of vitamin A ester, vitamin A alcohol or either of these plus tocopherols upon the vitamin A content of cow's colostrum following 4 weeks of pre-partal supplementation of the diet.

VITAMIN K

Meunier(303, 304), in organic chemical studies, confirms American findings that antivitamin K substances such as dicumarol, the active principle of spoiled sweet clover-hay, act only in the living animal and do not affect clotting of blood *in vitro*.

WATER-SOLUBLE VITAMINS

Bracken (*Pteris aquilina*) can be definitely added to those plant materials which are toxic, because they contain an antithiamine factor which in this case has been shown to be a thiaminase (Thomas & Walker(305), Evans & Evans(306)) thus amplifying earlier American work. Roberts, Evans

& Evans(307) have added the horse to animals subject to avitaminosis B_1 after ingesting bracken. Thiaminase has been reported in lamellibranchs (Fabriani, Fratoni & Spadoni(308)). Wells(309) reports a recent outbreak of bracken poisoning in cattle in England involving nine deaths. Stamp(310) has written a short but critical review of bracken poisoning in cattle obviously written before the role of vitamin B_1 in the syndrome was known over here(3). Haag & Weswig(311) have shown that the toxic factor is unstable to moist heat, while Evans and Evans have shown it to be a thiaminase and thus analogous to the Chastek enzyme(307,312). Austin(313) by balance experiments has shown that sheep are independent of a supply of the aneurin in their food and that the output of vitamin B_1 in the urine on certain diets is greater than the intake. Austin compared a stock ration, a synthetic diet free of vitamin B_1 and a diet consisting entirely of Moreton Bay fig tree leaves. The urinary output of vitamin B_1 varies with the nature of the diet. Three sheep were slaughtered after 60 days on the synthetic diet and analyses of rumen contents showed synthesis to be occurring in the rumen of each of the three sheep. Like other species the sheep requires more vitamin B_1 when pregnant. Unlike the rat, the sheep even if pregnant, on a vitamin B_1-deficient diet showed no adverse effects when given thyroxine. This, says Austin, is additional evidence of the sheep's independence of an external source of vitamin B_1.

Johnson, Hamilton, Nevens, Boley, Burke & Pinkos(314) fed fourteen newborn calves on a synthetic diet deficient in vitamin B_1. In addition to this diet, half the calves were given vitamin B_1. Those not receiving vitamin B_1 showed decreasing excretion of vitamin B_1 in the urine while pyruvate in blood and in urine rose. Typical symptoms of polyneuritis followed with muscular inco-ordination, scouring anorexia, head retraction and convulsions. Symptoms, except when accompanied by severe dehydration, were immediately relieved by aneurin therapy. Waugh, Hauge & King(315) found that amounts of choline chloride which may safely be given by mouth are quite toxic when injected.

Marsh, Pearson & Rupel(316) found that the feeding of synthetic thiamin, riboflavin, nicotinic acid and pantothenic acid at 2 g. per day to cows did not significantly affect the amount in the milk. Breed differences between Jerseys and Holsteins were found. In goats, the same four vitamins fed at 2 g. per day produced significant increases in amounts in milk, but increases in amounts in the urine were far greater still, even although only 10% of the dose fed was accounted for. What might be termed a metabiotic relationship has been exposed by the findings by Rubin & Bird(317) of chick growth factors in cow faeces which also contains male sex hormones(318).

Giannotti(319) found that supplements of yeast did not increase the fat content of cow's milk. McElroy, Rigney & Draper(320) have analysed many livestock feeds for choline.

WATER

Brody *et al.*(321) state that the first effect of heat on dairy cows is to cause an increase in their water intake. This agrees with the observations of Owen(11,322). In 1944, marked maxima in the urine volumes of cows were correlated with maxima of temperature of the environment. In 1948, cows whose heat burden increased as a result of thyroxine treatment showed much increased urine volumes. The cow's output of water in faeces, which are 85% water, and in the urine is quite big, so that the drinking of cold water which is voided at blood heat can considerably reduce the animal's heat burden. Brody(323) reports that the rise of rectal temperature of cows subjected to heat was least in those which drank most water. One cow increased her water consumption (with a corresponding increase of urine output) from 10 gal. at 50° F. to 45 gal. at 100° F. Clark & Quin(324) studied the water requirements of merino sheep and found that when hay and water are both fed *ad lib.* there was a significant positive correlation between their consumptions. There was between water consumption and the daily maximum temperature a similarly significant correlation which was enhanced by feeding a fixed amount of hay when the experiment was in summer but not during winter. These experiments were done in a typical inland warm temperate zone area with its usual accompaniment of summer rainfall so that the humidity was higher when the heat burden was greatest. When water was unrestricted there was no correlation between hay consumption and temperature, but when water was limited there was a highly significant

negative correlation between hay intake and temperature, presumably due to the extra heat load of post-prandial rumen fermentations. Animals deprived of water produced a concentrated urine containing 8 g. urea per 100 ml. In another paper (Clark & Quin(325)), in which summer and winter weather data are recorded, sheep were found to maintain their water intake equal to that taken *ad lib.* when watered for only 1 hr. on alternate days, but with water at 72 or 96 hr. intervals intake was decreased by 63 and 54% respectively. This restriction of water did not restrict grass hay intake, although one sheep died of dehydration. Lucerne hay intake was much depressed even by a 24 hr. thirst, presumably because the metabolic water produced by the digestion of lucerne would be much nearer to being isotonic with blood than would water from the digestion of grass hay. Nevertheless, sheep on lucerne hay and watered only twice weekly still gained weight, but the gain was accelerated by water *ad lib.* As exercise was greatly restricted these results cannot be directly extended to freely grazing sheep.

MINERALS

Ruminants have unusually large requirements of copper and cobalt. In sheep and cattle copper and cobalt deficiencies have a world-wide distribution and are largely determined either by the parent rocks from which soils are formed or by the nature of the soil. Soils deficient in cobalt produce pastures likewise deficient and on such pastures sheep and cattle become diseased. Extensively leached sedimentary soils such as those of coastal Western Australia or South Australia are prone to be both copper and cobalt deficient while neighbouring uplands with soil more recently formed from underlying lateritic rocks support healthy pastures. Reclaimed soils are apt, like the Dutch polders, to be copper deficient.

Cobalt

Cobalt deficiency has been reported in cattle from Denmark (Bendixen & Pedersen(326)), the Hebrides (Boddie(327)), in cattle and sheep in Ireland (Sheehy(328)), in Norway (Breirem(329)), in New Hampshire (Keener, Percival & Morrow(330)), and in sheep in Tasmania (Dumaresq & Wherrett(331)).

Working in South Australia, Marston, Lee & McDonald(332) studied the effects of known doses of copper and cobalt in sheep on land where 'coast disease' occurs. With 1 or 2 mg. cobalt and 10 mg. copper on 3 days a week sheep were kept in good health. Omission of either cobalt or copper caused typical symptoms of the respective deficiencies, the first symptom of copper deficiency being a change in the wool. Nickel, zinc, magnesium and iron conferred no extra benefit on animals receiving copper and cobalt. Copper- or cobalt-deficient animals had very small amounts of these elements in their livers. Ray, Weir, Pope & Phillips(333) found subnormal blood pyridoxine and nicotinic acid but normal riboflavine, folic acid and pantothenic acid in sheep maintained on diet containing cobalt-deficient hay.

It has not proved possible, by simple dietary restriction (Thompson & Ellis(334)), to demonstrate a requirement for cobalt except in the ruminant (Stewart(335)), and until quite recently there was no evidence as to whether non-ruminants required cobalt. Like the evidence for the mammal's requirement of zinc, which forms part of the enzyme carbonic anhydrase, the evidence for the essential nature of cobalt in non-ruminants has come from an unexpected quarter. Almost simultaneously in Britain and in the U.S.A. it has been shown that the antipernicious anaemia vitamin, B_{12}, is a compound of cobalt (Rickes, Brink, Koniuszy, Wood & Folkers(336); Smith(337)). It has further been shown that in certain sufferers from pernicious anaemia the curative dose of vitamin B_{12} is so minute that it would be impossible to devise a diet poor enough in cobalt to produce anaemia in a normal person. The ruminant needs cobalt for reasons other than to make vitamin B_{12} which, indeed, is normally present in cow manure (Littlee, Denton & Bird(338)). The only known resemblance beteen pining in sheep and pernicious anaemia in humans is that both are accompanied by anaemia. Marston & Lee(339) and Becker, Smith & Loosli(340) have shown that vitamin B_{12} has no effect on pining sheep whether injected or ingested. Tosic & Mitchell(341) showed that cobalt in the rumen is rapidly taken up by rumen bacteria, but the significance of this observation is

still not yet clear. Stewart(335) produced cobalt deficiency in lambs by feeding a ration providing 0·068 mg. per day. Ray, Weir, Pope, Bohstedt & Phillips(342), Marston & Lee(339) and Gall, Smith, Becker, Stark & Loosli(343) have shown that injected cobalt is ineffective as a cure for the pining of cobalt deficiency in sheep. Such injected cobalt accumulates in the liver, thus showing that liver cobalt is not *per se* a criterion of the adequacy of intake of cobalt, though it is probably valuable for diagnosis in the field (McNaught(344)). These recent experiments are consistent with the hypothesis that the site of action of cobalt is the rumen.

Comar(345) and Comar & Davis(346) investigated the metabolism of radioactive copper and cobalt in cattle, calves, rabbits and pigs.

Chamberlain, Branaman, Blakesless, Nelson & Thorp(347) found that ingestion of 100 mg. cobalt per day by an ewe caused the cobalt in milk to become 4 to 15 times bigger.

Pope, Phillips & Bohstedt(348), in North Wisconsin, found lack of thrift in lambs and sheep to be a cobalt-deficiency symptom. The giving of cobalt increased growth, and also vitamin A, vitamin C and haemoglobin in the blood. Rossiter, Curnow & Underwood(349) found that 30 g. $CoSO_4.7H_2O$ per acre per annum prevented sheep from pining on subterranean clover pastures. Ely, Duhn & Huffmann(350) and Kenner, Percival, Morrow & Ellis(351) found 40 or 50 mg. cobalt to be toxic to calves or young dairy cattle respectively. Symptoms of toxicity were decreased water intake and loss of appetite. In cattle large doses (100 mg.) caused inco-ordination. Seekles(352) quotes a report that cobalt activates diamine oxidases, e.g. histaminase, in the mucous membrane of the gut.

Copper

Copper is essential for the normal growth of wool (Marston & Hector(353); Marston & Lee(354)). Lee & Moule(355) suspected an area in Queensland of being copper deficient from the crimp of the wool. Copper treatment confirmed their diagnosis. Gerritsen(356) cured 'reclamation disease' of cattle in Holland by manuring the pastures with copper slag. Yield of pasture and of milk yields of cows improved dramatically, but the cows' blood copper responded only slowly. McDougall(357,358) has studied the distribution of copper concentration and total content of the foetal liver during the last 3 weeks pre-partum. The root cause of swayback in lambs, in spite of its prevention by copper, remains a mystery (Campbell, Daniel, Porter, Russel, Smith & Innes(359)). Comar, Davis & Singer(360) showed that Cu^{64} administered orally or by injection to bulls and bull calves was distributed to all tissues with a preference for the liver, especially in the very young animal. Marston & Lee(361) treated sheep of two different breeds with copper while they were depastured on a 'coasty' area in South Australia. Each received 1 mg. cobalt plus 0·1, 5, 50, or 100 mg. copper per day. Faulty myelination of nerves of their lambs was prevented by 5 but not by 1 mg. copper. The supplement of 100 mg. of copper produced haemorrhagic jaundice. British anti-lewisite (2:3-dimercaptopropanol) causes a much increased excretion of copper in the urine of the sheep (Stewart & Robertson(362)).

Hypocupremia

The well-known effect of copper by mouth or subcutaneously in inhibiting molybdenum scours (Green(363)) has been shown to have its analogue in rat metabolism (Comar, Singer & Davis(364)).

Efforts to demonstrate copper requirements of rumen bacteria *in vitro*(37) have not been successful, though the same set of experiments conclusively showed that the free iodophilic bacteria of the rumen require 2 p.p.m. of iron. In spite of the fact that swayback in sheep can be prevented by top-dressing pastures with copper, efforts to correlate the incidence of swayback with the copper content of pastures have not been successful, and the demyelination of nerve tracts of which swayback is the outward manifestation still has no simple explanation. There is indeed some doubt as to whether swayback is simply a copper deficiency(359).

Allcroft & Parker(365) have continued their investigations of the distribution of copper deficiency in cattle and sheep in Britain. Copper deficiency in cattle is a distinct entity from molybdenosis or 'teart' as it is called. Injected copper prevents molybdenum scours more effectively than ingested copper. Teart is, however, not simply copper deficiency, for there are farms in Britain where

deficiency of copper occurs in cattle uncomplicated by the presence of excessive molybdenum. Molybdenosis is probably not a disturbance of rumination (363, 364) and such a supposition is further supported by the observation of McNaught *et al.* (37) that molybdenum is relatively non-toxic to rumen bacteria growing *in vitro*.

Calcium and phosphorus

Ewer & Bartrum (366) report the occurrence of low phosphorus rickets in lambs feeding on young green oats or barley. Chemical analysis did not point to a deficiency of phosphorus or calcium. These authors suggest the presence of some substance, other than phytin, which inhibited phosphorus absorption. The condition was prevented or healed by single massive doses of vitamin D (10^6 i.u.) in the young sheep. Hodgson, Johnson, Wiese & Hickman (367) found that bullocks ate bone meal more readily than superphosphate and thrived better on either supplement than those not supplemented. Optimal thrift was, however, achieved only by a third group of bullocks to which the phosphatic supplements were force-fed.

Dennis & Harbaugh (368) found that tetany in cattle due to a large K/Ca in the blood resulted when KCl was fed, while $K_2C_2O_4$ was given intravenously. KCl fed alone or with K citrate did not induce tetany. Tetany occurred when K/Ca = 3·3. These experiments arose out of suspected poisoning of stock by excess K from grazing wheat.

Owen (286) has found that the negative calcium balance in the lactating cow is made even more negative by treatment with thyroxine, even although the food intake is increased so as to maintain the balance of nitrogen positive. In these experiments the effect of thyroxine was to produce a negative apparent digestibility of calcium or, in other words, to increase faecal metabolic calcium. Phosphorus balance was more positive during thyroxine treatment than either before or after treatment. Further experiments (Chanda & Owen (369); Chanda, McNaught & Owen (370)) make it probable that this retention of phosphorus is due to the effect of thyroxine in increasing the proportion of phosphoric acid bound in the various tissues of the body in ester form to facilitate transfers of energy in the tissues. These energy transfers constitute the increased metabolic rate for which the administration of thyroxine is responsible. Hansson (371), by excessive supplementation of rations with cobalt and phosphorus, found a decreased production in the high-yielding pairs and an increased production in low-yielding pairs.

Other minerals

Boddie (372) has reviewed fluorosis in domestic animals. Mather, Pratt & Holdaway (373) compared fused tricalcic phosphate containing 0·24% fluorine with two different levels of phosphate containing 2% fluorine as phosphatic supplements to calf starters and concentrates. Sacrificed at $3\frac{1}{2}$ years of age the animals showed no abnormality but had more fluorine in the bones with the bigger fluorine intake. At the higher intakes but not at the lower, teeth were abraded and discoloured. McConnel (374) has shown that selenium in the diet passes into casein, albumen and globulin of the milk.

Breirem, Ender, Halse & Slagsvold (375) investigated the metabolic effects of the cellulose-rich rations which, perforce, were fed to cows in Sweden during the war. The cellulose-rich ration was found to be responsible for a high incidence of hypomagnesemia. In a further experiment the cellulose-rich ration was compared with a sugar-rich ration and with one which was 20% deficient in energy. Hypomagnesemia again occurred on the cellulose-rich ration, but it produced no cases of ketosis. Ketosis was found paradoxically in three cows on the sugar ration and in cows on the hypocaloric ration. Hence ketosis is not necessarily preventable by giving sugar. A third experiment indicated that phosphorus, calcium, cobalt and carotene played no part in the genesis of ketosis. The hypomagnesemia was unaccompanied by clinical symptoms and thus resembled that earlier recorded by Allcroft (376), who described a seasonal rhythm in blood magnesium in Herefords which persisted when they were taken to a farm where Ayrshires showed no such rhythm. Allcroft therefore posits a hormonal control of blood magnesium; but the observations of Garner (377) that rumen organisms can readily liberate magnesium from grass powder, together

with the above experiments on cellulose-rich rations, make it more likely to be a simple reflexion by blood magnesium of the magnesium intake. Udall(378) found that parturient tetany accompanied by hypomagnesemia was cured by calcium gluconate but not by magnesium.

The dramatic effects of copper and cobalt in alleviating or preventing disease in sheep and cattle have diverted attention from the iron which was originally incriminated in these deficiencies. It is therefore interesting to see iron definitely implicated in the experiments on anaemia in calves by Jacobson & Moore(379). The anaemia could develop in calves of any age up to 60 days and slowly corrected itself thereafter. It was susceptible of immediate correction by administration of iron, but copper, cobalt and manganese were of no use. In view of the observation of McNaught *et al.*(37) that dietary iron becomes concentrated in rumen bacteria, which cannot multiply without it, it is possible that, on some diets, a transient anaemia due to iron deficiency develops in the calf during the time of rapid development of the rumen. Iron deficiency in cattle 9–18 months old has been reported by Gruningen, Keller & Pulver(380).

Axelsson & Kivimaë(381) added various mineral supplements to combat aciduria resulting from A.I.V. silage. None of their phosphatic supplements caused much change of the pH of the urine. The digestibilities of organic matter, crude fibre and crude protein were unaffected by the addition of $CaCO_3$. Organically bound silicon in some tissues of the calf has been reported by Ohlmeyer & Olpp(382). A survey of iodine content, and of histology and weight of thyroids, showed a widespread incidence of iodine-lack in newborn sheep (Andrews, Shrewsbury, Harper, Vestal & Doyle(383)) in Indiana. Data on the incidence of goitre in a wide variety of animals, including various ruminants, have been collected together(384). Bolin(385) records that 20 g. NaCl were lethal to a 60 lb. lamb, but that half this dose was tolerated. Manganese supplements made the negative calcium balances of dairy cows more negative, but improvement resulted when $CaCO_3$ was given(386). Ingested nickel, unlike cobalt, does not readily pass into the cow's milk(387).

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